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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 22221/1023 U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/048071	
INTERNATIONAL APPLICATION NO. PCT/US00/20666	INTERNATIONAL FILING DATE 28 July 2000 (28.07.00)	PRIORITY DATE CLAIMED 29 July 1999 (29.07.99)	
TITLE OF INVENTION DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS			
APPLICANT(S) FOR DO/EO/US O'DONNELL, Michael E.; BRUCK, Irina ; ZHANG, Dan and WHIPPLE, Richard			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11 to 20 below concern document(s) or information included:			
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PATENT

Docket No.: 22221/1023 (RU-429)

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Applicants	:	O'Donnell et al.)	Examiner:
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STATEMENT IN ACCORDANCE WITH 37 C.F.R. § 1.821(f)-(g)

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Respectfully submitted,

Dated: October 18, 2002


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**DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND
THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS**

5 The present application is a continuation-in-part of U.S. Patent
Application Serial No. 09/235,245 filed January 22, 1999, which claims benefit of
U.S. Provisional Patent Application Serial No. 60/093,727 filed July 22, 1998, and
U.S. Provisional Patent Application Serial No. 60/074,522 filed January 22, 1998, all
of which are hereby incorporated by reference. The present application also claims
benefit of U.S. Provisional Patent Application Serial No. 60/146,178 filed July 29,
10 1999, which is hereby incorporated by reference.

 The present invention was made with funding from National Institutes
of Health Grant No. GM38839. The United States Government may have certain
rights in this invention.

15 **FIELD OF THE INVENTION**

 This invention relates to genes and proteins that replicate the
chromosome of Gram positive bacteria. These proteins can be used in sequencing,
amplification of DNA, and in drug discovery to screen large libraries of chemicals for
20 identification of compounds with antibiotic activity.

BACKGROUND OF THE INVENTION

25 All forms of life must duplicate the genetic material to propagate the
species. The process by which the DNA in a chromosome is duplicated is called
replication. The replication process is performed by numerous proteins that
coordinate their actions to duplicate the DNA smoothly. The main protein actors are
as follows (reviewed in Kornberg et al., DNA Replication, Second Edition, New
York: W.H. Freeman and Company, pp. 165-194 (1992)). A helicase uses the energy
30 of ATP hydrolysis to unwind the two DNA strands of the double helix. Two copies of
the DNA polymerase use each "daughter" strand as a template to convert them into
two new duplexes. The DNA polymerase acts by polymerizing the four monomer unit
building blocks of DNA (the 4 dNTPs, or deoxynucleoside triphosphates are: dATP,
dCTP, dGTP, dTTP). The polymerase rides along one strand of DNA using it as a

template that dictates the sequence in which the monomer blocks are to be polymerized. Sometimes the DNA polymerase makes a mistake and includes an incorrect nucleotide (e.g., A instead of G). A proofreading exonuclease examines the polymer as it is made and excises building blocks that have been improperly inserted in the polymer.

Duplex DNA is composed of two strands that are oriented antiparallel to one another, one being oriented 3'-5' and the other 5' to 3'. As the helicase unwinds the duplex, the DNA polymerase moves continuously forward with the helicase on one strand (called the leading strand). However, due to the fact that DNA polymerases can only extend the DNA forward from a 3' terminus, the polymerase on the other strand extends DNA in the opposite direction of DNA unwinding (called the lagging strand). This necessitates a discontinuous ratcheting motion on the lagging strand in which the DNA is made as a series of Okazaki fragments. DNA polymerases cannot initiate DNA synthesis *de novo*, but require a primed site (i.e., a short duplex region). This job is fulfilled by primase, a specialized RNA polymerase, that synthesizes short RNA primers on the lagging strand. The primed sites are extended by DNA polymerase. A single-stranded DNA binding protein ("SSB") is also needed; it operates on the lagging strand. The function of SSB is to coat single stranded DNA ("ssDNA"), thereby melting short hairpin duplexes that would otherwise impede DNA synthesis by DNA polymerase.

The replication process is best understood for the Gram negative bacterium *Escherichia coli* and its bacteriophages T4 and T7 (reviewed in Kelman et al., "DNA Polymerase III Holoenzyme: Structure and Function of Chromosomal Replicating Machine," Annu. Rev. Biochem., 64:171-200 (1995); Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev. Biochem., 61:673-719 (1992); McHenry, C.S., "DNA Polymerase III Holoenzyme: Components, Structure, and Mechanism of a True Replicative Complex," J. Bio. Chem., 266:19127-19130 (1991); Young et al., "Structure and Function of the Bacteriophage T4 DNA Polymerase Holoenzyme," Am. Chem. Soc., 31:8675-8690 (1992)). The eukaryotic systems of yeast (*Saccharomyces cerevisiae*) (Morrison et al., "A Third Essential DNA Polymerase in *S. cerevisiae*," Cell, 62:1143-51 (1990) and humans (Bambara et al., "Reconstitution of Mammalian DNA Replication," Prog. Nuc. Acid Res., 51:93-123 (1995)) have also been characterized in some detail as has herpes virus (Boehmer et al., "Herpes

Simplex Virus DNA Replication," Annu. Rev. Biochem., 66:347-384 (1997)) and vaccinia virus (McDonald et al., "Characterization of a Processive Form of the Vaccinia Virus DNA Polymerase," Virology, 234:168-175 (1997)). The helicase of *E. coli* is encoded by the *dnaB* gene and is called the DnaB-helicase. In phage T4, the helicase is the product of the gene 41, and, in T7, it is the product of gene 4. Generally, the helicase contacts the DNA polymerase in *E. coli*. This contact is necessary for the helicase to achieve the catalytic efficiency needed to replicate a chromosome (Kim et al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996)). The identity of the helicase that acts at the replication fork in a eukaryotic cellular system is still not firm.

The primase of *E. coli* (product of the *dnaG* gene), phage T4 (product of gene 61), and T7 (gene 4) require the presence of their cognate helicase for activity. The primase of eukaryotes, called DNA polymerase alpha, looks and behaves differently. DNA polymerase alpha is composed of 4 subunits. The primase activity is associated with the two smaller subunits, and the largest subunit is the DNA polymerase which extends the product of the priming subunits. DNA polymerase alpha does not need a helicase for priming activity on single strand DNA that is not coated with binding protein.

The chromosomal replicating DNA polymerase of all these systems, prokaryotic and eukaryotic, share the feature that they are processive, meaning they remain continuously associated with the DNA template as they link monomer units (dNTPs) together. This catalytic efficiency can be manifest *in vitro* by their ability to extend a single primer around a circular ssDNA of over 5,000 nucleotide units in length. Chromosomal DNA polymerases will be referred to here as replicases to distinguish them from DNA polymerases that function in other DNA metabolic processes and are far less processive.

There are three types of replicases known thus far that differ in how they achieve processivity and how their subunits are organized. These will be referred to here as Types I-III. The Type I is exemplified by the phage T5 replicase, which is composed of only one subunit yet is highly processive (Das et al., "Mechanism of Primer-template Dependent Conversion of dNTP-dNMP by T7 DNA Polymerase," J. Biol. Chem., 255:7149-7154 (1980)). It is possible that the T5 enzyme achieves

processivity by having a cavity within it for binding DNA, with a domain of the protein acting as a lid that opens to accept the DNA and closes to trap the DNA inside, thereby keeping the polymerase on DNA during polymerization of dNTPs. Type II is exemplified by the replicases of phage T7, herpes simplex virus, and vaccinia virus.

5 In these systems, the replicase is composed of two subunits, the DNA polymerase and an "accessory protein" which is needed for the polymerase to become highly efficient. It is presumed that the DNA polymerase binds the DNA in a groove and that the accessory protein forms a cap over the groove, trapping the DNA inside for processive action. Type III is exemplified by the replicases of *E. coli*, phage T4, yeast, and
10 humans in which there are three separate components, a sliding clamp protein, a clamp loader protein complex, and the DNA polymerase. In these systems, the sliding clamp protein is an oligomer in the shape of a ring. The clamp loader is a multiprotein complex which uses ATP to assemble the clamp around DNA. The DNA polymerase then binds the clamp which tethers the polymerase to DNA for high
15 processivity. The replicase of the *E. coli* system contains a fourth component called tau that acts as a glue to hold two polymerases and one clamp loader together into one structure called Pol III*. In this application, any replicase that uses a minimum of three components (i.e., clamp, clamp loader, and DNA polymerase) will be referred to as either a three component polymerase, a type III enzyme, or a DNA polymerase III-
20 type replicase.

The *E. coli* replicase is also called DNA polymerase III holoenzyme. The holoenzyme is a single multiprotein particle that contains all the components; it is comprised of ten different proteins. This holoenzyme is suborganized into four functional components called: 1) Pol III core (DNA polymerase); 2) gamma complex
25 or tau/gamma complex (clamp loader); 3) beta subunit (sliding clamp); and 4) tau (glue protein). The DNA polymerase III "core" is a tightly associated complex containing one each of the following three subunits: 1) the alpha subunit is the actual DNA polymerase (129 kDa); 2) the epsilon subunit (28 kDa) contains the proofreading 3'-5' exonuclease activity; and 3) the theta subunit has an unknown
30 function. The gamma complex is the clamp loader and contains the following subunits: gamma, delta, delta prime, chi and psi (U.S. Patent No. 5,583,026 to O'Donnell). Tau can substitute for gamma, as can a tau/gamma heterooligomer. The beta subunit is a homodimer and forms the ring shaped sliding clamp. These

components associate to form the holoenzyme and the entire holoenzyme can be assembled *in vitro* from 10 isolated pure subunits (U.S. Patent No. 5,583,026 to O'Donnell; U.S. Patent No. 5,668,004 to O'Donnell). The *E. coli dnaX* gene encodes both tau and gamma. Tau is the product of the full gene. Gamma is the product of the first 2/3 of the gene; it is truncated by an efficient translational frameshift that results in incorporation of one unique residue followed by a stop codon.

The tau subunit, encoded by the same gene that encodes gamma (*dnaX*), also acts as a glue to hold two cores together with one gamma complex. This subassembly is called DNA polymerase III star (Pol III*). One beta ring interacts with each core in Pol III* to form DNA polymerase III holoenzyme.

During replication, the two cores in the holoenzyme act coordinately to synthesize both strands of DNA in a duplex chromosome. At the replication fork, DNA polymerase III holoenzyme physically interacts with the DnaB helicase through the tau subunit to form a yet larger protein complex termed the "replisome" (Kim et al., "Coupling of a Replicative Polymerase and Helicase: A tau-DnaB Interaction Mediates Rapid Replication Fork Movement," Cell, 84:643-650 (1996); Yuzhakov et al., "Replisome Assembly Reveals the Basis for Asymmetric Function in Leading and Lagging Strand Replication," Cell, 86:877-886 (1996)). The primase repeatedly contacts the helicase during replication fork movement to synthesize RNA primers on the lagging strand (Marians, K.J., "Prokaryotic DNA Replication," Annu. Rev. Biochem., 61:673-719 (1992)).

Intensive subtyping of prokaryotic cells has now lead to a taxonomic classification of prokaryotic cells as eubacteria (true bacteria) to distinguish them from archaeobacteria. Within eubacteria are many different subcategories of cells, although they can broadly be subdivided into Gram positive - and Gram negative-like cells. Numerous complete and partial genome sequences of prokaryotes have appeared in the public databases.

In the present invention, new genes from the Gram positive bacteria, *Streptococcus pyogenes* (e.g., *S. pyogenes*) and *Staphylococcus aureus* (e.g., *S. aureus*) are identified. They are assigned names based on their nearest homology to subunits in the *E. coli* system. The genes encoding *E. coli* replication proteins are as follows: alpha (*dnaE*); epsilon (*dnaQ*); theta (*holE*); tau (full length *dnaX*); gamma

(frameshift product of *dnaX*); delta (*holA*); delta prime (*holB*); chi (*holC*); psi (*holD*); beta (*dnaN*); DnaB helicase (*dnaB*); and primase (*dnaG*).

Study of the organisms for which a complete genome sequence is available reveals that no organism has identifiable homologues to all the subunits of the *E. coli* three component polymerase, Pol III holoenzyme (see Table 1 below). All other organisms lack the θ subunit (*holE*), and all except one lack genes encoding the χ and ψ subunits (*holC* and *holD*, respectively) as judged by sequence comparison searches. Further, the α and ϵ subunits are fused into one large α subunit in some organisms (e.g., Gram positive cells) as detailed in (Sanjanwala et al., "DNA Polymerase III Gene of *Bacillus subtilis*," Proc. Natl. Acad. Sci., USA, 86:4421-4424 (1989)). Although all organisms have homologues to τ , β , δ' and SSB, the δ subunit has diverged significantly (either not recognized or nearly not recognized by gene searching programs), perhaps even to the point where it is no longer involved in DNA replication. The DnaX product also would appear to lack frameshift signals in most organisms. This predicts only one protein (tau) will be produced from this gene, instead of two as in *E. coli*. Indeed, this has been shown to be true for the *Staphylococcus aureus* DnaX (U.S. Patent Application Serial No. 09/235,245, which is hereby incorporated by reference). Finally, genetic study of *Bacillus subtilis* identified two genes that do not have counterparts in *E. coli* (*dnaB*, not the helicase, and *dnaH*) as well as one other gene, *dnaI*, that is only very distantly related to *E. coli dnaC* (Karamata et al., "Isolation and Genetic Analysis of Temperature-Sensitive Mutants of *B. subtilis* Defense in DNA Synthesis," Molec. Gen. Genet., 108:277-287 (1970); Braund et al., "Nucleotide Sequence of the *Bacillus subtilis dnaD* Gene," Microb., 141:321-322 (1995); Hoshino et al., "Nucleotide Sequence of *Bacillus subtilis dnaB*: A Gene Essential for DNA Replication Initiation and Membrane Attachment," Proc. Natl. Acad. Sci. USA," 84:653-657 (1987)). Keeping in mind the apparently random, or at least unpredictable process of evolution, it is possible that these apparently new genes perform novel functions that may result in a new type of polymerase for chromosomal replication. Thus, it seems possible that new proteins may have evolved to take the place of χ , ψ , θ , the frameshift product of DnaX, and possibly δ in other eubacteria. These considerations indicate that the three component polymerase of different eubacteria may have different structures. That this may be so would not be surprising as different bacteria are often less related evolutionarily than plants are to

humans. For example, the split between Gram positive and Gram negative bacteria occurred about 1.2 billion years ago. This distant split makes Gram positive cells an attractive source to examine how different other eubacterial three component polymerases are from the *E. coli* Pol III holoenzyme.

Table 1

Organism (Order)	χ	ψ	θ	ϵ	α	β	<u>dnaX</u>	δ'	δ
<i>Escherichia coli</i> Proteobacteria	+	+	+	+	+	+	+	+	+
<i>Haemophilus influenzae</i> Proteobacteria	+	+	—	+	+	+	+	+	+
<i>Mycoplasma genitalium</i> Firmicutes	—	—	—	—	+	+	+	+	+
<i>Synichisystis sp.</i> Cyanobacteria	—	—	—	—	+	+	+	+	+
<i>Bacillus subtilis</i> Firmicutes	—	—	—	—	+	+	+	+	+
<i>Borrelia burgdorferi</i> Spirochaetales	—	—	—	—	+	+	+	+	+
<i>Aquifex aeolicus</i> Aquificales	—	—	—	+	+	+	+	+	+
<i>Mycobacterium tuberculosis</i> Firmicutes & Actinobacteria	—	—	—	+	+	+	+	+	+
<i>Treponema pallidum</i> Spirochaetales	—	—	—	+	+	+	+	+	+
<i>Chlamydia trachomatis</i> Chlamydiales	—	—	—	+	+	+	+	+	+
<i>Rickettsia prowazekii</i> Proteobacteria	—	—	—	+	+	+	+	+	+
<i>Helicobacter pylori</i> Proteobacteria	—	—	—	+	+	+	+	+	+
<i>Thermatoga maritima</i> Thermotogales	—	—	—	—	+	+	+	+	+

5

The goal of this invention is to learn how to form a functional three component polymerase from an organism that is highly divergent from *E. coli* and whether it is as rapid and processive as the *E. coli* Pol III holoenzyme. Namely, from bacteria lacking χ , ψ , or θ , or having a widely divergent δ subunit, or having only one DnaX product, or an α subunit that encompasses both α and ϵ activities. All eubacteria for which the entire genome has been sequenced have at least one of these differences from *E. coli*. Many Gram negative bacteria have one or more of these differences (e.g., *Haemophilus influenzae* and *Aquifex aeolicus*). Bacteria of the Gram positive class have all of these different features. Because of the distant

10

evolutionary split between Gram positive and Gram negative bacteria, their mechanisms of replication may have diverged significantly as well. Indeed, purification of the replication polymerase from *B. subtilis*, a Gram positive cell, gives only a single subunit polymerase (Barnes et al., "Purification of DNA Polymerase III of Gram-Positive Bacteria," Methods Enzy. 262:35-42 (1995); Barnes et al., "Antibody to *B. subtilis* DNA Polymerase III: Use in Enzyme Purification and Examination of Homology Among Replication-specific DNA Polymerases," Nucl. Acids Res., 6:1203-209 (1979); Barnes et al., "DNA Polymerase III of *Mycoplasma pulmonis*: Isolation and Characterization of the Enzyme and its Structural Gene, *polC*," Mol. Microb., 13:843-854, (1994); Low et al., "Purification and Characterization of DNA Polymerase III from *Bacillus subtilis*," J. Biol. Chem., 251:1311-1325 (1976)) instead of a 10 subunit assembly containing the three components of a rapidly processive machine as discussed above for Pol III holoenzyme from *E. coli*. This finding suggests a different structural organization of the replicase and possibly different functional characteristics as well.

Although there are many studies of replication mechanisms in eukaryotes and, specifically, the Gram negative bacterium *E. coli* and its bacteriophages, there is very little information about how Gram positive organisms replicate. The Gram positive class of bacteria includes some of the worst human pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis* (Youmans et al., The Biological and Clinical Basis of Infectious Disease (1985)). Until this invention, the best characterized Gram positive organism for chromosomal DNA synthesis was *Bacillus subtilis*. Fractionation of *B. subtilis* has identified three DNA polymerases. (Gass et al., "Further Genetic and Enzymological Characterization of the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," J. Biol. Chem., 248:7688-7700 (1973); Ganesan et al., "DNA Replication in a Polymerase I Deficient Mutant and the Identification of DNA Polymerases II and III in *Bacillus subtilis*," Biochem. Biophys. Res. Commun., 50:155-163 (1973)). These polymerases are thought to be analogous to the three DNA polymerases of *E. coli* (DNA polymerases I, II, and III). Studies in *B. subtilis* have identified a polymerase that appears to be involved in chromosome replication and is termed Pol III (Ott et al., "Cloning and Characterization of the *polC* Region of *Bacillus subtilis*," J. Bacteriol., 165:951-957 (1986); Barnes et al.,

“Localization of the Exonuclease and Polymerase Domains of *Bacillus subtilis* DNA Polymerase III,” Gene, 111:43-49 (1992); Barnes et al., “The 3’-5’ Exonuclease Site of DNA Polymerase III From Gram-positive Bacteria: Definition of a Novel Motif Structure,” Gene” 165:45-50 (1995) or Barnes et al., “Purification of DNA

5 Polymerase III of Gram-positive Bacteria,” Methods in Enzy., 262:35-42 (1995)). The *B. subtilis* Pol III (encoded by *polC*) is larger (about 165 kDa) than the *E. coli* alpha subunit (about 129 kDa) and exhibits 3’-5’ exonuclease activity. The *polC* gene encoding this Pol III shows weak homology to the genes encoding *E. coli* alpha and the *E. coli* epsilon subunit. Hence, this long form of the *B. subtilis* Pol III (herein

10 referred to as α -large or Pol III-L) essentially comprises both the alpha and epsilon subunits of the *E. coli* core polymerase. The *S. aureus* α -large has also been sequenced, expressed in *E. coli*, and purified; it contains DNA polymerase and 3’-5’ exonuclease activity (Pacitti et al., “Characterization and Overexpression of the Gene Encoding *Staphylococcus aureus* DNA Polymerase III,” Gene, 165:51-56 (1995)).

15 Although α -large is essential to cell growth (Clements et al., “Inhibition of *Bacillus subtilis* Deoxyribonucleic Acid Polymerase III by Phenylhydrazinopyrimidines: Demonstration of a Drug-induced Deoxyribonucleic Acid-Enzyme Complex,” J. Biol. Chem., 250:522-526 (1975); Cozzarelli et al., “Mutational Alteration of *Bacillus subtilis* DNA Polymerase III to Hydroxyphenylazopyrimidine Resistance: Polymerase

20 III is Necessary for DNA Replication,” Biochem. And Biophy. Res. Commun., 51:151-157 (1973); Low et al., “Mechanism of Inhibition of *Bacillus subtilis* DNA Polymerase III by the Arylhydrazinopyrimidine Antimicrobial Agents,” Proc. Natl. Acad. Sci. USA, 71:2973-2977 (1974)), there could still be another DNA

25 polymerase(s) that is essential to the cell, such as occurs in yeast (Morrison et al., “A Third Essential DNA Polymerase in *S. cerevisiae*,” Cell, 62:1143-1151 (1990)).

Purification of α -large from *B. subtilis* results in only this single protein without associated proteins (Barnes et al., “Localization of the Exonuclease and Polymerase Domains of *Bacillus subtilis* DNA Polymerase III,” Gene, 111:43-49 (1992); Barnes et al., “The 3’-5’ Exonuclease Site of DNA Polymerase III From

30 Gram-positive Bacteria: Definition of a Novel Motif Structure,” Gene” 165:45-50 (1995) or Barnes et al., “Purification of DNA Polymerase III of Gram-positive Bacteria,” Methods in Enzymol., 262:35-42 (1995)). Hence, it is possible that α -large is a member of the Type I replicase (like T5) in which it is processive on its own

without accessory proteins. *B. subtilis* and *S. aureus* also have a gene encoding a protein that has approximately 30% homology to the beta subunit of *E. coli*; however, the protein product has not been purified or characterized (Alonso et al., "Nucleotide Sequence of the *recF* Gene Cluster From *Staphylococcus aureus* and
5 Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 246:680-686 (1995); Alonso et al., "Nucleotide Sequence of the *recF* Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet., 248:635-636 (1995)). Whether this beta subunit has a function in replication, a ring shape, or functions as a sliding clamp was not known
10 until recently. It was also not known whether it is functional with α -large. Recently, it was shown that *S. aureus* β is functional as a ring, and that it also functions with α -large (U.S. Patent Application Serial No. 09/235,245, which is hereby incorporated by reference). Further, a fourth DNA polymerase was identified with greater homology to *E. coli* α than α -large. This polymerase, called herein α -small, is shorter than α -
15 large and lacks the domain homologous to epsilon. This polymerase also functions with the β ring, indicating that it may participate in chromosome replication. Indeed, a recent report indicates that α -small is essential for replication in *Streptomyces coelicolor* A3(2) (Flett et al., "A Gram-negative type' DNA Polymerase III is Essential for Replication of the Linear Chromosome of *Streptomyces Coelicolor* A3(2)," Mol.
20 Micro., 31:949-958, (1999)).

As described earlier, purification of the replicase from the Gram positive *B. subtilis* gives only a single subunit Pol III, instead of a multicomponent complex. Also, *S. aureus dnaX* has been shown to encode only one subunit (U.S. Patent Application Serial No. 09/235,245, which is hereby incorporated by reference).
25 Moreover, *S. aureus* and *B. subtilis* lack homologues to χ , ψ , θ , and the δ subunit is only weakly homologous to δ of *E. coli* (only 28%). Further, they lack a homologue to *dnaQ* encoding ϵ . Instead, they contain this activity (3'-5' exonuclease) in the *polC* gene product which provides the α -large form of α . The ϵ subunit is needed for high speed and processivity of the *E. coli* Pol III holoenzyme; the α subunit alone is much
30 less rapid and processive with the β ring compared to the presence of both α and ϵ (Studwell et al., "Processive Replication is Contingent on the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol Chem., 265: 1171-1178 (1990)).

Studies using the *E. coli* β ring (and γ complex) show they confer onto *S. aureus* α quite efficient synthesis (U.S. Patent Application Serial No. 09/235,245, which is hereby incorporated by reference), but the efficiency is not equal to that of *E. coli* $\alpha\epsilon$ with β (and γ complex). This may be due to use of the heterologous combination of an α subunit from one organism (*S. aureus*) with the β clamp from another (*E. coli*). However, it is also possible that *S. aureus* α simply does not function with a β clamp to produce speed and processivity comparable to the *E. coli* polymerase. Also, as described earlier, the α -large subunit of *B. subtilis* purifies as a single subunit, rather than associated with accessory subunits assembled into the three components of a rapid, processive machine (i.e., like *E. coli* Pol III holoenzyme). The lack of two DnaX products, lack of a multicomponent structure, and lack of gene homologues encoding several subunits of the three component, Pol III, of *E. coli* brings into question whether other types of bacteria, such as Gram positive cells, even have an enzyme with similar structure or comparable speed and processivity to that found in the Gram negative *E. coli*.

The lack of gene homologues encoding several subunits of the *E. coli* three component polymerase creates uncertainties with respect to reconstructing a rapid and processive polymerase from a Gram positive cell that has characteristics like the Pol III system of *E. coli*.

The γ and δ' proteins are homologous to one another, encoding C-shape proteins (Dong et al., "DNA Polymerase III Accessory Proteins," J. Biol. Chem., 268:11758-11765, (1993); Guenther et al., "Crystal Structure of the δ' Subunit of the Clamp-loader Complex of *E. coli* DNA Polymerase III," Cell, 91:335-345 (1997)). The clamp loaders of yeast and humans are composed of five proteins, all of which are homologous to one another and to γ and δ' (Cullman et al., "Characterization of the Five Replication Factor C Genes of *Saccharomyces Cerevisiae*," Mol. Cell. Biol., 15:4661-4671 (1995)). This provides evidence that a clamp loader can be composed entirely of C-shape proteins. Perhaps the Gram positive DnaX-protein (hereafter referred to as τ) and δ' are sufficient to provide function as a clamp loader. Indeed, the clamp loader of T4 phage is composed of only two different proteins, gp44/62 complex (Young et al., "Structure and Function of the Bacteriophage T4 DNA Polymerase Holoenzyme," Biochem., 31:8675-8690 (1992)). This idea is also

supported by the presence of only two RFC genes in archaeobacteria, suggesting that they may utilize two C-shaped proteins for clamp loading, in contrast to yeast and humans that use five. With this consideration in mind, genes were identified and isolated and the τ protein (encoded by *dnaX*) and δ' (encoded by *holB*) of another
5 Gram positive organism, *Streptococcus pyogenes*, were expressed and purified. As was observed in *S. aureus*, *S. pyogenes dnaX* produces only a single polypeptide. The β , encoded by *dnaN* of *S. pyogenes*, was also identified, expressed, and purified, as were the α -large subunit encoded by *polC* and the SSB encoded by the *ssb* gene. These proteins were studied for interactions and characterized for their effect on α -
10 large. However, the hypothesis was incorrect as τ and δ' did not form a $\tau\delta'$ complex, nor did they assemble β onto DNA or provide stimulation of α when using β on primed and SSB coated M13mp18 ssDNA.

In light of the inability of *S. pyogenes* τ protein and δ' to function as a clamp loader, it seemed reasonable to expect that one or more other proteins are
15 needed. The fact that *E. coli* has some replicase subunits that other bacteria do not, suggests that other bacteria may have some replicase subunits that *E. coli* does not. Indeed, genetic studies of *Bacillus subtilis* demonstrates that it has three genes needed for replication that *E. coli* does not have. Two of these novel genes, called *dnaB* (not the same as *E. coli dnaB* encoding the helicase) and *dnaH*, have no significant
20 homology to genes in the *E. coli* genome database (Bruand et al., "Nucleotide Sequence of the *Bacillus subtilis dnaD* gene," Microbiol., 141:321-322 (1995); Hoshino et al., "Nucleotide Sequence of *Bacillus subtilis dnaB*: A gene Essential for DNA replication Initiation and Membrane Attachment," Proc. Natl. Acad. Sci. USA, 84:653-657 (1987)). Further, *dnaI* of *B. subtilis* is important for replication and has, at best, a very limited homology to *E. coli dnaC* (Karamata et al., "Isolation and
25 Genetic Analysis of Temperature-Sensitive Mutants of *B. subtilis* Defective in DNA synthesis," Molec. Gen. Genetics, 108:277-287 (1970)). Perhaps one or more of these genes encode the proteins(s) necessary to provide clamp loading activity when combined with τ and δ' , or to couple with α to provide it with speed and/or
30 processivity as the *E. coli* epsilon does. The *S. pyogenes* homologues of *B. subtilis dnaI*, *dnaH*, and *dnaB* were identified, cloned, and the encoded proteins were expressed and purified. However, these proteins failed to provide activity alone or in

combinations with *S. pyogenes* τ and δ' in loading *S. pyogenes* β onto DNA, or in stimulating *S. pyogenes* α -large in combination with β , τ , and δ' on SSB coated primed M13mp18 ssDNA.

Weak homology exists for the *holA* gene among prokaryotes. This weak homologue of *holA* was identified in *S. pyogenes* and, then, it was cloned, expressed, and the putative δ was purified. The putative δ formed an isolatable complex with τ and δ' . In fact, the $\tau\delta\delta'$ complex loaded *S. pyogenes* β onto DNA, and it stimulated *S. pyogenes* α -large in a β dependent reaction on primed SSB coated M13mp18 ssDNA. Hence, this protein was the only missing component necessary to provide clamp loading activity. Further, a mixture of α with $\tau\delta\delta'$, followed by ion exchange chromatography on MonoQ, indicated formation of an $\alpha\tau\delta\delta'$ complex. Consistent with this, τ appeared to bind α in gel filtration analysis.

Whether the *S. pyogenes* three component polymerase can synthesize DNA in as rapid and processive of a fashion as the *E. coli* Pol III holoenzyme three component polymerase is very difficult to predict, because no other DNA polymerase known to date catalyzes synthesis at the rate or processivity of the *E. coli* three component polymerase. For example, the three component T4 phage polymerase travels about 400 nucleotides/s, the yeast DNA polymerase delta three component polymerase travels about 120 nucleotides/s, and the human DNA polymerase delta three component enzyme appears slower and less processive than the yeast enzyme.

The standard test for these speed and processivity characteristics is examination of a time course in extension of a primer on a very long template, such as around the 7.2 kb M13mp18 ssDNA genome coated with SSB and primed with a synthetic DNA oligonucleotide. The results of experiments of this type demonstrate that the three component *S. pyogenes* polymerase is indeed extremely rapid in synthesis. Surprisingly, it is just as fast as the *E. coli* enzyme. Extension proceeds at about 700-800 nucleotides per second, completing the entire template in about 9 seconds. The enzyme was fully processive throughout replication of the M13mp18 genome, as could be determined from the fact that some templates were not extended at all, while others were extended to completion. If the enzyme had not been processive during the entire replication reaction, then when it comes off one partially extended DNA genome it would have reassociated with the unextended DNA that

remained and partially replicated it as well (and so on until the entire population of DNA became fully replicated). This did not happen. Instead, the reaction showed a mixture of completely replicated templates and templates that were still untouched starting material. This indicates that the enzyme stays with a template until it
5 completes it before it cycles over to replicate another one (i.e., it is highly processive). Each of the five proteins, α , τ , δ , δ' and β , are needed to obtain this rapid and processive DNA synthesis.

This invention has provided an intellectual template by which the clamp loader component of these three component polymerases can be obtained from
10 any eubacterial prokaryotic cell and how to use it with the other components to produce a rapid and processive polymerase. All prokaryotes in the eubacterial kingdom that have been sequenced to date contain homologues of these genes. As the process of lateral gene transfer appears to be a major force in evolution, it would appear that relatedness of enzymes and enzyme machines is best judged by
15 comparisons of their genes and proteins rather than by phylogeny of which bacteria they are in (Doolittle et al., "Archaeal Genomics: Do Archaea have a Mixed Heritage?," Curr. Biol., 8:R209-R211 (1998)). As pointed out earlier in this application, most bacteria have genetic characteristics of replication genes/proteins of *S. pyogenes* rather than that of *E. coli* (i.e., no genes encoding χ , ψ , or θ , only a weak
20 homolog to δ , or a *dnaX* gene encoding only a single protein).

The *dnaX* gene encoding τ and γ in *E. coli* encodes only one protein in some organisms, but, as this application shows, it is still functional in forming a protein complex capable of rapid and processive DNA synthesis. In addition, this application shows that the delta subunit, which is only weakly homologous among
25 different prokaryotic organisms, is an essential functional subunit of the three component polymerase (instead of having diverged so as to fulfill an entirely different function in some other intracellular process). As mentioned earlier, several genes encoding subunits of the *E. coli* clamp loader (γ complex; γ , δ , δ' , χ , ψ) are not obviously present in other prokaryotes (*holC* and *holD* encoding χ and ψ). Hence, one may
30 anticipate that other genes may have evolved to encode new subunits that replace these, and that these new subunits may have been essential to the activity of the clamp loader. For example, they may have either taken over some of the functionality of

another subunit, or structurally (e.g., the physical presence of a subunit could be needed for one subunit to assume its proper and active conformation, or for one or more of the subunits to form a complex together to yield the multisubunit clamp loader assembly). In addition, this application shows that the α subunit (*polC* gene product) is sufficient for rapid and processive synthesis with the other two components (i.e., *E. coli* requires ϵ subunit to bind to α for rapid and processive synthesis of α with the β clamp). Finally, this application shows that the *S. pyogenes* three component polymerase synthesizes DNA as fast as the *E. coli* Pol III three component polymerase. Up to this point, the *E. coli* Pol III three component polymerase was over twice the speed of the T4 enzyme and over 5 times the speed of others. Hence, it was possible that *E. coli* may have been unique among prokaryotes in having a polymerase that achieves such speed. This invention shows that this is not the case. Instead, this speed in polymerization generalizes to the Gram positive prokaryotic three component DNA polymerases. It may be presumed, now that two examples of three component polymerases in widely divergent bacteria share the characteristics of rapid, processive synthesis, that the three component polymerase of other eubacteria will also be rapid and processive.

These rapid and processive three component DNA polymerases can be applied to several important uses. DNA polymerases currently in use for DNA sequencing and DNA amplification use enzymes that are much slower and thus could be improved upon. This is especially true of amplification as the three component polymerase is capable of speed and high processivity making possible amplification of very long (tens of Kb to Mb) lengths of DNA in a time efficient manner. These three component polymerases also function in conjunction with a replicative helicase (DnaB) and, thus, are capable of amplification at ambient temperature using the helicase to melt the DNA duplex. This property could be useful in amplification reaction procedures such as in polymerase chain reaction (PCR) methodology. Finally, these three component polymerases and their associated helicase (DnaB) and primase (DnaG) are attractive targets for antibiotics due to their essential and central role in cell viability.

This application provides a three component polymerase from two human pathogens in the Gram positive class. It makes possible the production of this three component polymerase from other bacteria of the Gram positive type (e.g.,

Streptococci, *Staphylococci*, *Mycoplasma*) and other types of bacteria lacking χ , ψ , or θ , those having only one protein produced by their *dnaX* gene such as obligate intracellular parasites, Mycoplasmas (possibly evolved from Gram positives), Cyanobacteria (*Synechocystis*), Spirochaetes such as *Borrelia* and *Treponema* and *Chlamydia*, and distant relatives of *E. coli* in the Gram negative class (e.g., *Rickettsia* and *Helicobacter*). These three component polymerases are useful in manipulation of nucleic acids for research and diagnostic purposes (e.g., sequencing and amplification methods) and for screening chemicals for antibiotic activity (useful in human or animal therapy and agriculture such as animal feed supplements). There are several assays described previously in U.S. Patent Application Serial No. 09/235,245 to O'Donnell et al., which is hereby incorporated by reference, that use these three component polymerases (or subassemblies), as well as the DnaB and DnaG homologues, either alone or in various combinations, for the purpose of screening chemicals, such as chemical libraries, for inhibitor activity. Such inhibitors can be developed further (usually by chemical manipulation and alteration) into lead compounds and then into full fledged pharmaceuticals.

There remains a need to understand the molecular details of the process of DNA replication in other cells that are quite different from *E. coli*, such as in Gram positive cells. It is possible that a more detailed understanding of replication proteins will lead to discovery of new antibiotics. Therefore, a deeper understanding of replication proteins of Gram positive bacteria is especially important given the emergence of drug resistant strains of these organisms. For example, *Staphylococcus aureus* has successfully mutated to become resistant to all common antibiotics.

The "target" protein(s) of an antibiotic drug is generally involved in a critical cell function, such that blocking its action with a drug causes the pathogenic cell to die or no longer proliferate. Current antibiotics are directed to very few targets. These include membrane synthesis proteins (e.g., vancomycin, penicillin, and its derivatives such as ampicillin, amoxicillin, and cephalosporin), the ribosome machinery (e.g., tetracycline, chloramphenicol, azithromycin, and the aminoglycosides such as kanamycin, neomycin, gentamicin, streptomycin), RNA polymerase (e.g., rifampimycin), and DNA topoisomerases (e.g., novobiocin, quinolones, and fluoroquinolones). The DNA replication apparatus is a crucial life process and, thus, the proteins involved in this process are good targets for antibiotics.

A powerful approach to discovery of a new drug is to obtain a target protein, characterize it, and develop *in vitro* assays of its cellular function. Large chemical libraries can then be screened in the functional assays to identify compounds that inhibit the target protein. These candidate pharmaceuticals can then be

5 chemically modified to optimize their potency, breadth of antibiotic spectrum, non-toxicity, performance in animal models and, finally, clinical trials. The screening of large chemical libraries requires a plentiful source of the target protein. An abundant supply of protein generally requires overproduction techniques using the gene encoding the protein. This is especially true for replication proteins as they are

10 present in low abundance in the cell.

Selective and robust assays are needed to screen reliably a large chemical library. The assay should be insensitive to most chemicals in the concentration range normally used in the drug discovery process. These assays should also be selective and not show inhibition by antibiotics known to target proteins in

15 processes outside of replication.

The present invention is directed to overcoming these deficiencies in the art.

SUMMARY OF THE INVENTION

20 The present invention relates to various isolated DNA molecules from *Staphylococcus aureus* and *Streptococcus pyogenes*, both of which are Gram positive bacteria. These include DNA molecules which include a coding region from the *dnaE* gene (encoding α - small), *dnaX* gene (encoding tau), *polC* gene (encoding Pol III –L

25 or α - large), *dnaN* gene (encoding beta), *holA* gene (encoding delta), *holB* gene (encoding delta prime), *ssb* gene (encoding SSB), *dnaB* gene (encoding DnaB), and *dnaG* gene (encoding DnaG) of *S. aureus* and/or *S. pyogenes*. These DNA molecules can be inserted into an expression system and used to transform host cells. The isolated proteins or polypeptides encoded by these DNA molecules, and their ability to

30 function when used in combination is also disclosed. The resulting actions provide assembling a ring onto DNA via a clamp loader, and polymerase activity dependent on this ring that is rapid and processive.

A further aspect of the present invention relates to a method of identifying compounds which inhibit activity of a polymerase product of *polC* or *dnaE*. This method is carried out by forming a reaction mixture comprising a primed DNA molecule, a polymerase product of *polC* or *dnaE*, a candidate compound, a dNTP, and optionally either a beta subunit, a tau complex, or both the beta subunit and the tau complex, wherein at least one of the polymerase product of *polC* or *dnaE*, the beta subunit, the tau complex, or a subunit or combination of subunits thereof is derived from a Eubacteria other than *Escherichia coli*; subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound; analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and identifying the candidate compound in the reaction mixture where there is an absence of nucleic acid polymerization extension products.

The present invention deciphers the structure and mechanism of the chromosomal replicase of Gram positive bacteria and other bacteria lacking *holC*, *holD*, *holE* or *dnaQ* genes, or having a *dnaX* gene that encodes only one protein. Rather than use a DNA polymerase that attains high efficiency on its own, or with one other subunit, the Gram positive bacteria replicase is a three component type of replicase (class III) that uses a sliding clamp protein. The Gram positive bacteria replicase also uses a clamp loader component that assembles the sliding clamp onto DNA. This knowledge, and the enzymes involved in the replication process, can be used for the purpose of screening for potential antibiotic drugs. Further, information about chromosomal replicases may be useful in DNA sequencing, DNA amplification, polymerase chain reaction, and other DNA polymerase related techniques.

The present invention identifies two DNA polymerases (both of Pol III type) in Gram positive bacteria that utilize the sliding clamp and clamp loader. The present invention also identifies a gene with homology to the alpha subunit of *E. coli* DNA polymerase III holoenzyme, the chromosomal replicase of *E. coli*. These DNA polymerases can extend a primer around a large circular natural template when the beta clamp has been assembled onto the primed ssDNA by the clamp loader or a primer on a linear DNA where the beta clamp may assemble by itself by sliding over an end.

The present invention shows that the clamp and clamp loader components of Gram negative cells can be exchanged for those of Gram positive cells in that the clamp, once assembled onto DNA, will function with Pol III obtained from either Gram positive and Gram negative sources. This result implies that important contacts between the polymerase and clamp have been conserved during evolution. Therefore, these "mixed systems" may provide assays for an inhibitor of this conserved interaction. Such an inhibitor may be expected to shut down replication, and since the interaction is apparently conserved across the evolutionary spectrum from Gram positive and Gram negative cells, the inhibitor may exhibit a broad spectrum of antibiotic activity.

The present invention demonstrates that Gram positive bacteria contain a beta subunit that behaves as a sliding clamp that encircles DNA. A *dnaX* gene sequence encoding a protein homolog of the gamma/tau subunit of the clamp loader (gamma/tau complex) *E. coli* DNA polymerase III holoenzyme is also identified. The presence of this gene confirms the presence of a clamp loading apparatus in Gram positive bacteria that will assemble beta clamps onto DNA for the DNA polymerases.

This application also outlines methods and assays for use of these replication proteins in drug screening processes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the construction of the *S. aureus* Pol III-L expression vector. The gene encoding Pol III-L was cloned into a pET11 expression vector in a three step cloning scheme as illustrated.

Figures 2A-C describe the expression and purification of *S. aureus* Pol III-L (alpha-large). Figure 2A compares *E. coli* cells that contain the pET11PolC expression vector that are either induced or uninduced for protein expression. The gel is stained with Coomassie Blue. The induced band corresponds to the expected molecular weight of the *S. aureus* Pol III-L, and is indicated to the right of the gel. Figure 2B shows the results of the MonoQ chromatography of a lysate of *E. coli* (pET11PolC-L) induced for Pol III-L. The fractions were analyzed in a Coomassie Blue stained gel (top) and for DNA synthesis (bottom). Fractions containing Pol III-L are indicated. In Figure 2C, fractions containing Pol III-L from the MonoQ column

were pooled and chromatographed on a phosphocellulose column. This shows an analysis of the column fractions from the phosphocellulose column in a Coomassie Blue stained polyacrylamide gel. The position of Pol III-L is indicated to the right.

Figure 3 shows the *S. aureus* beta expression vector. The *dnaN* gene was amplified from *S. aureus* genomic DNA and cloned into the pET16 expression vector.

Figures 4A-C illustrate the expression and purification of *S. aureus* beta. Figure 4A compares *E. coli* cells that contain the pET16beta expression vector that are either induced or uninduced for protein expression. The gel is stained with Coomassie Blue. The induced band corresponds to the expected molecular weight of the *S. aureus* beta, and is indicated to the right of the gel. The migration position of size standards are indicated to the left. Figure 4B shows the results of MonoQ chromatography of an *E. coli* (pET16beta) lysate induced for beta. The fractions were analyzed in a Coomassie Blue stained gel, and fractions containing beta are indicated. In Figure 4C, fractions containing beta from the MonoQ column were pooled and chromatographed on a phosphocellulose column. This shows an analysis of the column fractions from the phosphocellulose column in a Coomassie Blue stained polyacrylamide gel. The position of beta is indicated to the right.

Figures 5A-B demonstrate that the *S. aureus* beta stimulates *S. aureus* Pol III-L and *E. coli* Pol III core on linear DNA, but not circular DNA. In Figure 5A, the indicated proteins were added to replication reactions containing polydA-oligodT as described in the Examples *infra*. Amounts of proteins added, when present, were: lanes 1,2: *S. aureus* Pol III-L, 7.5 ng; *S. aureus* beta, 6.2 µg; Lanes 3,4: *E. coli* Pol III core, 45 ng; *S. aureus* beta, 9.3 µg; Lanes 5,6: *E. coli* Pol III core, 45 ng; *E. coli* beta, 5µg. Total DNA synthesis was: Lanes 1-6: 4.4, 30.3, 5.1, 35.5, 0.97, 28.1 pmol, respectively. In Figure 5B, Lanes 1-3, the indicated proteins were added to replication reactions containing circular singly primed M13mp18 ssDNA as described in the Examples *infra*. *S. aureus* beta, 0.8 µg; *S. aureus* Pol III-L, 300 ng (purified through MonoQ); *E. coli* clamp loader complex, 1.7 µg. Results in the *E. coli* system are shown in Lanes 4-6. Total DNA synthesis was: Lanes 1-6: 0.6, 0.36, 0.99, 2.7, 3.5, 280 pmol, respectively.

Figure 6 shows that *S. aureus* Pol III-L functions with *E. coli* beta and clamp loader complex on circular primed DNA. It also shows that *S. aureus* beta does

not convert Pol III-L with sufficient processivity to extend the primer all the way around a circular DNA. Replication reactions were performed on the circular singly primed M13mp18 ssDNA. Proteins added to the assay are as indicated in this figure. The amount of each protein, when present, is: *S. aureus* beta, 800 ng; *S. aureus* Pol III-L, 1500 ng (MonoQ fraction 64); *E. coli* Pol III core, 450 ng; *E. coli* beta, 100 ng; *E. coli* gamma complex, 1720 ng. Total DNA synthesis in each assay is indicated at the bottom of the figure.

Figures 7A-B show that *S. aureus* contains four distinct DNA polymerases. Four different DNA polymerases were partially purified from *S. aureus* cells. *S. aureus* cell lysate was separated from DNA and, then, chromatographed on a MonoQ column. Fractions were analyzed for DNA polymerase activity. Three peaks of activity were observed. The second peak was the largest and was expected to be a mixture of two DNA polymerases based on early studies in *B. subtilis*. Chromatography of the second peak on phosphocellulose (Figure 7B) resolved two DNA polymerases from one another.

Figures 8A-B show that *S. aureus* has two DNA Pol III's. The four DNA polymerases partially purified from *S. aureus* extract, designated peaks I-IV in Figure 7, were assayed on circular singly primed M13mp18 ssDNA coated with *E. coli* SSB either in the presence or absence of *E. coli* beta (50ng) and clamp loader complex (50 ng). Each reaction contained 2 µl of the partially pure polymerase (Peak 1 was Mono Q fraction 24 (1.4 µg), Peak 2 was phosphocellulose fraction 26 (0.016 mg/ml), Peak 3 was phosphocellulose fraction 46 (0.18 mg/ml), and Peak 4 was MonoQ fraction 50 (1 µg). Figure 8A shows the product analysis in an agarose gel. Figure 8B shows the extent of DNA synthesis in each assay.

Figure 9 compares the homology between the polypeptide encoded by *dnaE* of *S. aureus* and other organisms. An alignment is shown for the amino acid sequence of the *S. aureus dnaE* product with the *dnaE* products (alpha subunits) of *E. coli* and *Salmonella typhimurium*.

Figure 10 compares the homology between the N-terminal regions of the gamma/tau polypeptides of *S. aureus*, *B. subtilis*, and *E. coli*. The conserved ATP site and the cystines forming the zinc finger are indicated above the sequence. The organisms used in the alignment were: *E. coli* (GenBank); and *B. subtilis*.

Figure 11 compares the homology between the DnaB polypeptide of *S. aureus* and other organisms. The organisms used in the alignment were: *E. coli* (GenBank); *B. subtilis*; *Sal. Typ.*, (*Salmonella typhimurium*).

Figures 12A-B show the alignment of the delta subunit encoded by *hola* for *E. coli* and *B. subtilis* (Figure 12A) and for the delta subunit of *B. subtilis* and *S. pyogenes* (Figure 12B). Figure 12A shows ClustalW generated alignment of *S. pyogenes* (Gram positive) delta to *E. coli* (Gram negative) delta. Figure 12B shows ClustalW generated alignment of *B. subtilis* (Gram positive) delta to *S. pyogenes* (Gram positive) delta.

Figure 13 is an image of an autoradiograph of an agarose gel analysis of replication products from singly primed, SSB coated M13mp18 ssDNA using the reconstituted *S. aureus* Pol III holozyeme. Only in the presence of the $\tau\delta\delta'$ complex does α -large (PolC) function with β to replicate a full circular duplex DNA (RFII).

Figure 14 shows a Comassie Blue stained SDS polyacrylamide gel of the pure *S. pyogenes* subunits corresponding to alpha-large, alpha-small, *dnaX* gene product (called tau), beta, delta, delta prime, and SSB. The first lane shows the position of molecular weight markers. Purified proteins were separated on a 15% SDS-PAGE and stained with Coomassie Brilliant Blue R-250. Each lane contains 5 microgram of each protein. Lane 1, markers; lane 2, alpha-large; lane 3, alpha-small, lane 4, tau subunit; lane 5, beta subunit; lane 6, delta subunit; lane 7, delta prime subunit; lane 8, single strand DNA binding protein.

Figures 15A-C document the ability to reconstitute the $\tau\delta\delta'$ complex of *S. pyogenes*. Proteins were mixed and gel filtered on Superose 6, followed by analysis of the column fractions in a SDS polyacrylamide gel. Figure 15A shows a mixture of $\tau\delta\delta'$. Figure 15B shows a mixture of $\tau\delta$. Figure 15C shows a mixture of $\tau\delta'$.

Figures 16A-E show that the *S. pyogenes* $\tau\delta\delta'$ complex can load the *S. pyogenes* beta clamp onto (circular) DNA. Loading reactions contained 500 fm nicked pBSK plasmid, 500 fm either $\tau\delta\delta'$ complex, tau, delta, or delta prime, 1pm ^{32}P -labelled beta dimer, 8 mM MgCl_2 , 1 mM ATP. Reaction components were preincubated for 10 min at 37°C prior to loading onto 5 ml Biogel A15M column equilibrated with buffer A containing 100 mM NaCl. Figure 16A demonstrates the ability of $\tau\delta\delta'$ complex to load the beta dimer onto a nicked pBSK circular plasmid.

Figures 16B-E show the results of using either: beta alone (Figure 16B); $\delta\delta'$ plus β (Figure 16C); τ , δ and β (Figure 16D); τ , δ' and β (Figure 16E).

Figures 17A-C show that τ and alpha interact. Figure 17A shows the result of gel filtration analysis of a mixture of τ with alpha-large. Gel filtration fractions are analyzed in a SDS polyacrylamide gel. Figures 17B and 17C show the results using only τ or only alpha-large, respectively. Comparison of the elution positions of proteins shows that the positions of alpha and tau are shifted toward a higher molecular weight complex when they are present together. The fact they do not exactly comigrate may indicate that they initially are together in a complex, but that the complex dissociates during the time of the gel filtration experiment (over one half hour).

Figures 18A-B document the ability to reconstitute $\alpha_L\tau\delta\delta'$ (pol III*) complex of *S. pyogenes*. Proteins were mixed, preincubated for 20 min at 15°C, gel filtered on Superose 6, followed by analysis of the column fractions in a SDS polyacrylamide gel (Figure 18A). Proteins were loaded on a MonoQ column, then eluted with a linear gradient of 50-500 mM NaCl, followed by analysis of the column fractions in a SDS polyacrylamide gel (Figure 18B). The $\alpha_L\tau\delta\delta'$ complex migrates early.

Figure 19 illustrates the speed and processivity of the *S. pyogenes* $\alpha_L\tau\delta\delta'$ (pol III*) complex. The $\alpha_L\tau\delta\delta'$ (pol III*) complex was incubated with primed M13pm18 ssDNA (coated with *S. pyogenes* SSB) and only two dNTPs, then replication was initiated upon adding the remaining two dNTPs. Reactions contained 25 fmol singly primed M13mp18 ssDNA template, 300 fmol β_2 , and either 75 fmol or 250 fmol $\alpha_L\tau\delta\delta'$. Time points were quenched with SDS/EDTA then analyzed in a neutral agarose gel followed by autoradiography. Each time point is a separate reaction. The time course of polymerization was performed at two different ratios of polymerase/primed template to assess speed and processivity of nucleotide incorporation.

Figures 20A-I show the extent of homology between *S. pyogenes* replication genes and other organisms. Due to the low homology of delta (Figure 20D), one must "walk" from one organism to the next in order to recognize the homologue with high probability. Percent identity over regions of the indicated

number of amino acid residues is shown for each match (i.e., the two organisms at the opposite ends of each line). Amino acid sequences were retrieved from either GenBank or individual unfinished genome databases.

Figure 21A-F are images illustrating that the *S. pyogenes* DnaE (alpha-small) polymerase functions with β . Figures 21A-B illustrate the relationship between DnaE and β for association with ssDNA. Different amounts of DnaE polymerase were added to a SSB coated M13mp18 ssDNA circle primed with a single DNA oligonucleotide, and products were analyzed in a native agarose gel. Reactions were performed in the presence of $\tau\delta\delta'$ and either the absence (Figure 21C, panels 1-4) or presence (Figure 21D, panels 1-4) of β . Positions of completed duplex (RFII) and initial primed template (ssDNA) are indicated. Figure 21E shows an analysis of exonuclease activity by PolC and DnaE on a 5'-32P-DNA 30-mer. Aliquots were removed at the indicated times and analyzed in a sequencing gel. Figure 21F shows the effect of TMAU on PolC and DnaE in the presence of $\tau\delta\delta'$ and β . DNA products were analyzed in a native agarose gel. Positions of initial primed M13mp18 (ssDNA) and completed circular duplex (RFII) are indicated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to various isolated nucleic acid molecules from Gram positive bacteria and other bacteria lacking *holC*, *holD*, or *holE* genes or having a *dnaX* gene encoding only one subunit. These include DNA molecules which correspond to the coding regions of the *dnaE*, *dnaX*, *holA*, *holB*, *polC*, *dnaN*, *SSB*, *dnaB*, and *dnaG* genes. These DNA molecules can be inserted into an expression system or used to transform host cells. The isolated proteins or polypeptides encoded by these DNA molecules and their use to form a three component polymerase are also disclosed. Also encompassed by the present invention are corresponding RNA molecules transcribed from the DNA molecules.

These DNA molecules and proteins can be derived from numerous bacteria, including *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Mycoplasma*, *Mycobacterium*, *Borrelia*, *Treponema*, *Rickettsia*, *Chlamydia*, *Helicobacter*, and *Thermatoga*. It is particularly directed to such DNA molecules and proteins derived from *Streptococcus* and *Staphylococcus* bacteria, particularly *Streptococcus pyogenes*.

and *Staphylococcus aureus* (see U.S. Patent Application Serial No. 09/235,245, which is hereby incorporated by reference).

The gene sequences used to obtain DNA molecules of the present invention were obtained by sequence comparisons with the *E. coli* counterparts, followed by detailed analysis of the raw sequence data in the contigs from the *S. pyogenes* database (<http://dna1.chem.ou.edu/strep.html>) or the *S. aureus* database (<http://www.genome.ou.edu/staph.html>) to identify the open reading frames. In many instances, nucleotide errors were observed causing frameshifts in the open reading frame thus truncating it. Therefore, upon cloning the genes via PCR, the genes were sequenced to obtain correct information. Also, the full nucleotide sequence of the *ssb* gene was not present in the data base. This was cloned by circular PCR and the full sequence is reported below.

The *S. aureus dnaX* and *dnaE* genes were identified by aligning genes of several organisms and designing primers for use in PCR to obtain a gene fragment, followed by steps to identify the entire gene.

One aspect of the present invention relates to a newly discovered Pol III gene (herein identified as *dnaE*) of *S. aureus* whose encoded protein is homologous to *E. coli* alpha (product of *dnaE* gene). The partial nucleotide sequence of the *S. aureus dnaE* gene corresponds to SEQ. ID. No. 1 as follows:

```

atggtggcat atttaaatat tcatacggct tatgatttgt taaattcaag cttaaaaaata 60
gaagatgccg taagacttgc tgtgtctgaa aatgttgatg cacttgccat aactgacacc 120
aatgtattgt atggttttcc taaattttat gatgcatgta tagcaaataa cattaaaccg 180
atttttggta tgacaatata tgtgacaaat ggattaaata cagtcgaaac agttgttcta 240
gctaaaaata atgatggatt aaaagatttg tatcaactat catcggaat aaaaatgaat 300
gcattagaac atgtgtcggt tgaattatta aaacgatttt ctaacaatat gattatcatt 360
tttaaaaaag tcggtgatca acatcgtgat attgtacaag tgtttgaaac ccataatgac 420
acatatatgg accaccttag tatttcgatt caaggtagaa aacatgtttg gattcaaaat 480
gtttgttacc aaacacgtca agatgccgat acgattttctg cattagcagc tattagagac 540
aatacaaaat tagacttaat tcatgatcaa gaagattttg gtgcacattt tttaaactgaa 600
aaggaaatta atcaattaga tattaaccaa gaatatttaa cgcaggttga tggtatagct 660
caaaagtgtg atgcagaatt aaaatatcat caatctctac ttctcaata tgagacacct 720
aatgatgaat cagctaaaaa atattttgtgg cgtgtcttag ttacacaatt gaaaaaatta 780
gaacttaatt atgacgtcta tttagagcga ttgaaatatg agtataaagt tattactaat 840
atggggtttg aagattattt cttaaatagta agtgatttaa tccattatgc gaaaacgaat 900
gatgtgatgg taggtcctgg tegtgttctc tcagctggct cactggtcag ttatttattg 960
ggaattacaa cgattgatcc tattaaattc aatctattat ttgaacgttt tttaaaccca 1020
gaacgtgtaa caatgcctga tattgatatt gactttgaag atacacgccg agaaaggggc 1080
attcagtacg tccaagaaaa atatggcgag ctacatgtat ctggaattgt gactttcggt 1140
catctgcttg caagagcagt tgctagagat gttggaagaa ttatgggggt tgatgaagt 1200
acattaaatg aaatttcaag tttaatccca cataaattag gaattacact tgatgaagca 1260
tatcaaattg acgattttta agagtttgta catcgaaacc atcgacatga acgctgggtc 1320
agtatttgta aaaagttaga aggtttacca agacatacat ctacacatgc ggcaggaatt 1380
attattaatg accatccatt atatgaatat gcccttttaa cgaaagggga tacaggatta 1440
ttaacgcaat ggacaatgac tgaagccgaa cgtattgggt tattaaaaat agattttcta 1500
gggttgagaa acttatcgat tattcatcaa atcttaacac aagtcaaaaa agatttaggt 1560

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5   attaatatg atatcgaaaa gattccggtt gatgatcaaa aagtgtttga attgttgtcg 1620
    caaggagata cgactggcat attccaatta gagtctgacg gtgtaagaag tgtattaaaa 1680
    aaattaaagc cggaacactt tgaagatatt gttgctgtaa cttctttgta tagaccaggt 1740
    ccaatggaag aaattccaac ttacattaca agaagacatg atccaagcaa agttcaatat 1800
    ttacatccgc atttagaacc tatattaaaa aatacttacg gtgttattat ttatcaagag 1860
    caaattatgc aaatagcgag cacatttgca aacttcagtt atgggtgaagc ggatatttta 1920
    agaagagcaa tgagtaaaaa aaatagagct gttcttgaaa gtgagcgtca acattttata 1980
    gaaggtgcaa agcaaaatgg ttatcacgaa gacattagta agcaaatatt tgatttgatt 2040
10  ctgaaatttg ctgattatgg ttttcctaga gcacatgctg tcagctattc taaaattgca 2100
    tacattatga gctttttaaa agtccattat ccaaattatt tttacgcaaa tattttaagt 2160
    aatgttattg gaagtgaagaa gaaaactgct caaatgatag aagaagcaaa aaaacaaggt 2220
    atcactatat tgccaccgaa cattaacgaa agtcattgggt tttataaacc ttccaagaa 2280
    ggcattttatt tatcaattgg tacaattaaa ggtgttgggt atcaaagtgt gaaagtgatt 2340
    gttgatgaac gttatcagaa cggcaaattt aaagatttct ttgattttgc tagacgtata 2400
15  ccgaagagag tcaaaacgag aaagttaact gaagcactga ttttagtggg agcgtttgat 2460
    gcttttggtg aaacacgttc aacgttgttg caagctattg atcaagtgtt ggatggcgat 2520
    ttaaacattg aacaagatgg ttttttattt gatattttta cgccaaaaca gatgtatgaa 2580
    gataaagaag aattgcctga tgcacttatt agtcagtagc aaaaagaata tttaggattt 2640
    tatgtttcgc aacacccagt agataaaaag tttgttgcca aacaatattt acgatatttt 2700
20  aaattgagtc agcgcgagaa ttataaacct atattagtag agtttgataa agttaaacta 2760
    attcgaacta aaaatgggtc aaatatggca ttcgtcacat taaatgatgg cattgaaact 2820
    ttagatgggt tgattttccc taatcagttt aaaaagtacg aagagttggt atcacataat 2880
    gacttgttta tagtttagcg gaaatttgac catagaaagc aacaacgtca actaattata 2940
    aatgagattc agacattagc cacttttgaa gaacaaaaat tagcatttgc caaacaattt 3000
25  ataattagaa ataaatcaca aatagatag tttgaagaga tgattaaagc tacgaaagag 3060
    aatgctaata atgttgtgtt atccttttat gatgaaacga ttaaacaaat gactacttta 3120
    ggctatatta atcaaaaaga tagtatgttt aataatttta tacaatcctt taaccttagt 3180
    gatattaggc ttata                                     3195

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30 The *S. aureus dnaE* encoded protein, called α -small, has an amino acid sequence corresponding to SEQ. ID. No. 2 as follows:

```

35  Met Val Ala Tyr Leu Asn Ile His Thr Ala Tyr Asp Leu Leu Asn Ser
     1          5          10          15
    Ser Leu Lys Ile Glu Asp Ala Val Arg Leu Ala Val Ser Glu Asn Val
          20          25          30
    Asp Ala Leu Ala Ile Thr Asp Thr Asn Val Leu Tyr Gly Phe Pro Lys
          35          40          45
    Phe Tyr Asp Ala Cys Ile Ala Asn Asn Ile Lys Pro Ile Phe Gly Met
          50          55          60
45  Thr Ile Tyr Val Thr Asn Gly Leu Asn Thr Val Glu Thr Val Val Leu
     65          70          75          80
    Ala Lys Asn Asn Asp Gly Leu Lys Asp Leu Tyr Gln Leu Ser Ser Glu
          85          90          95
50  Ile Lys Met Asn Ala Leu Glu His Val Ser Phe Glu Leu Leu Lys Arg
     100          105          110
    Phe Ser Asn Asn Met Ile Ile Ile Phe Lys Lys Val Gly Asp Gln His
     115          120          125
    Arg Asp Ile Val Gln Val Phe Glu Thr His Asn Asp Thr Tyr Met Asp
     130          135          140
60  His Leu Ser Ile Ser Ile Gln Gly Arg Lys His Val Trp Ile Gln Asn
     145          150          155          160

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	Val	Cys	Tyr	Gln	Thr	Arg	Gln	Asp	Ala	Asp	Thr	Ile	Ser	Ala	Leu	Ala	
					165					170					175		
5	Ala	Ile	Arg	Asp	Asn	Thr	Lys	Leu	Asp	Leu	Ile	His	Asp	Gln	Glu	Asp	
				180					185					190			
	Phe	Gly	Ala	His	Phe	Leu	Thr	Glu	Lys	Glu	Ile	Asn	Gln	Leu	Asp	Ile	
			195					200					205				
10	Asn	Gln	Glu	Tyr	Leu	Thr	Gln	Val	Asp	Val	Ile	Ala	Gln	Lys	Cys	Asp	
		210					215					220					
15	Ala	Glu	Leu	Lys	Tyr	His	Gln	Ser	Leu	Leu	Pro	Gln	Tyr	Glu	Thr	Pro	
	225					230					235					240	
	Asn	Asp	Glu	Ser	Ala	Lys	Lys	Tyr	Leu	Trp	Arg	Val	Leu	Val	Thr	Gln	
					245					250					255		
20	Leu	Lys	Lys	Leu	Glu	Leu	Asn	Tyr	Asp	Val	Tyr	Leu	Glu	Arg	Leu	Lys	
				260					265					270			
	Tyr	Glu	Tyr	Lys	Val	Ile	Thr	Asn	Met	Gly	Phe	Glu	Asp	Tyr	Phe	Leu	
			275					280					285				
25	Ile	Val	Ser	Asp	Leu	Ile	His	Tyr	Ala	Lys	Thr	Asn	Asp	Val	Met	Val	
		290					295					300					
30	Gly	Pro	Gly	Arg	Gly	Ser	Ser	Ala	Gly	Ser	Leu	Val	Ser	Tyr	Leu	Leu	
	305					310					315					320	
	Gly	Ile	Thr	Thr	Ile	Asp	Pro	Ile	Lys	Phe	Asn	Leu	Leu	Phe	Glu	Arg	
					325					330					335		
35	Phe	Leu	Asn	Pro	Glu	Arg	Val	Thr	Met	Pro	Asp	Ile	Asp	Ile	Asp	Phe	
				340					345					350			
	Glu	Asp	Thr	Arg	Arg	Glu	Arg	Val	Ile	Gln	Tyr	Val	Gln	Glu	Lys	Tyr	
			355					360					365				
40	Gly	Glu	Leu	His	Val	Ser	Gly	Ile	Val	Thr	Phe	Gly	His	Leu	Leu	Ala	
		370					375					380					
45	Arg	Ala	Val	Ala	Arg	Asp	Val	Gly	Arg	Ile	Met	Gly	Phe	Asp	Glu	Val	
	385					390					395					400	
	Thr	Leu	Asn	Glu	Ile	Ser	Ser	Leu	Ile	Pro	His	Lys	Leu	Gly	Ile	Thr	
					405					410					415		
50	Leu	Asp	Glu	Ala	Tyr	Gln	Ile	Asp	Asp	Phe	Lys	Glu	Phe	Val	His	Arg	
				420					425					430			
	Asn	His	Arg	His	Glu	Arg	Trp	Phe	Ser	Ile	Cys	Lys	Lys	Leu	Glu	Gly	
			435					440					445				
55	Leu	Pro	Arg	His	Thr	Ser	Thr	His	Ala	Ala	Gly	Ile	Ile	Ile	Asn	Asp	
		450					455					460					
60	His	Pro	Leu	Tyr	Glu	Tyr	Ala	Pro	Leu	Thr	Lys	Gly	Asp	Thr	Gly	Leu	
	465					470					475					480	
	Leu	Thr	Gln	Trp	Thr	Met	Thr	Glu	Ala	Glu	Arg	Ile	Gly	Leu	Leu	Lys	
					485					490						495	

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	Ile	Asp	Phe	Leu	Gly	Leu	Arg	Asn	Leu	Ser	Ile	Ile	His	Gln	Ile	Leu
				500					505					510		
5	Thr	Gln	Val	Lys	Lys	Asp	Leu	Gly	Ile	Asn	Ile	Asp	Ile	Glu	Lys	Ile
			515					520					525			
	Pro	Phe	Asp	Asp	Gln	Lys	Val	Phe	Glu	Leu	Leu	Ser	Gln	Gly	Asp	Thr
		530					535					540				
10	Thr	Gly	Ile	Phe	Gln	Leu	Glu	Ser	Asp	Gly	Val	Arg	Ser	Val	Leu	Lys
	545				550						555					560
	Lys	Leu	Lys	Pro	Glu	His	Phe	Glu	Asp	Ile	Val	Ala	Val	Thr	Ser	Leu
15					565					570					575	
	Tyr	Arg	Pro	Gly	Pro	Met	Glu	Glu	Ile	Pro	Thr	Tyr	Ile	Thr	Arg	Arg
				580					585					590		
20	His	Asp	Pro	Ser	Lys	Val	Gln	Tyr	Leu	His	Pro	His	Leu	Glu	Pro	Ile
			595					600					605			
	Leu	Lys	Asn	Thr	Tyr	Gly	Val	Ile	Ile	Tyr	Gln	Glu	Gln	Ile	Met	Gln
		610					615					620				
25	Ile	Ala	Ser	Thr	Phe	Ala	Asn	Phe	Ser	Tyr	Gly	Glu	Ala	Asp	Ile	Leu
	625					630					635					640
	Arg	Arg	Ala	Met	Ser	Lys	Lys	Asn	Arg	Ala	Val	Leu	Glu	Ser	Glu	Arg
30					645					650					655	
	Gln	His	Phe	Ile	Glu	Gly	Ala	Lys	Gln	Asn	Gly	Tyr	His	Glu	Asp	Ile
				660					665					670		
35	Ser	Lys	Gln	Ile	Phe	Asp	Leu	Ile	Leu	Lys	Phe	Ala	Asp	Tyr	Gly	Phe
			675					680					685			
	Pro	Arg	Ala	His	Ala	Val	Ser	Tyr	Ser	Lys	Ile	Ala	Tyr	Ile	Met	Ser
		690					695					700				
40	Phe	Leu	Lys	Val	His	Tyr	Pro	Asn	Tyr	Phe	Tyr	Ala	Asn	Ile	Leu	Ser
	705					710					715					720
	Asn	Val	Ile	Gly	Ser	Glu	Lys	Lys	Thr	Ala	Gln	Met	Ile	Glu	Glu	Ala
45					725					730					735	
	Lys	Lys	Gln	Gly	Ile	Thr	Ile	Leu	Pro	Pro	Asn	Ile	Asn	Glu	Ser	His
				740					745					750		
50	Trp	Phe	Tyr	Lys	Pro	Ser	Gln	Glu	Gly	Ile	Tyr	Leu	Ser	Ile	Gly	Thr
			755					760					765			
	Ile	Lys	Gly	Val	Gly	Tyr	Gln	Ser	Val	Lys	Val	Ile	Val	Asp	Glu	Arg
		770					775					780				
55	Tyr	Gln	Asn	Gly	Lys	Phe	Lys	Asp	Phe	Phe	Asp	Phe	Ala	Arg	Arg	Ile
	785					790					795					800
	Pro	Lys	Arg	Val	Lys	Thr	Arg	Lys	Leu	Leu	Glu	Ala	Leu	Ile	Leu	Val
60					805					810					815	
	Gly	Ala	Phe	Asp	Ala	Phe	Gly	Lys	Thr	Arg	Ser	Thr	Leu	Leu	Gln	Ala
				820					825						830	

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	Ile	Asp	Gln	Val	Leu	Asp	Gly	Asp	Leu	Asn	Ile	Glu	Gln	Asp	Gly	Phe	
			835					840					845				
5	Leu	Phe	Asp	Ile	Leu	Thr	Pro	Lys	Gln	Met	Tyr	Glu	Asp	Lys	Glu	Glu	
		850					855					860					
	Leu	Pro	Asp	Ala	Leu	Ile	Ser	Gln	Tyr	Glu	Lys	Glu	Tyr	Leu	Gly	Phe	
	865					870					875					880	
10	Tyr	Val	Ser	Gln	His	Pro	Val	Asp	Lys	Lys	Phe	Val	Ala	Lys	Gln	Tyr	
				885						890					895		
	Leu	Thr	Ile	Phe	Lys	Leu	Ser	Asn	Ala	Gln	Asn	Tyr	Lys	Pro	Ile	Leu	
15				900					905					910			
	Val	Gln	Phe	Asp	Lys	Val	Lys	Gln	Ile	Arg	Thr	Lys	Asn	Gly	Gln	Asn	
		915						920					925				
20	Met	Ala	Phe	Val	Thr	Leu	Asn	Asp	Gly	Ile	Glu	Thr	Leu	Asp	Gly	Val	
	930						935					940					
	Ile	Phe	Pro	Asn	Gln	Phe	Lys	Lys	Tyr	Glu	Glu	Leu	Leu	Ser	His	Asn	
	945					950					955					960	
25	Asp	Leu	Phe	Ile	Val	Ser	Gly	Lys	Phe	Asp	His	Arg	Lys	Gln	Gln	Arg	
				965						970					975		
	Gln	Leu	Ile	Ile	Asn	Glu	Ile	Gln	Thr	Leu	Ala	Thr	Phe	Glu	Glu	Gln	
30				980					985					990			
	Lys	Leu	Ala	Phe	Ala	Lys	Gln	Ile	Ile	Ile	Arg	Asn	Lys	Ser	Gln	Ile	
		995						1000					1005				
35	Asp	Met	Phe	Glu	Glu	Met	Ile	Lys	Ala	Thr	Lys	Glu	Asn	Ala	Asn	Asp	
	1010						1015					1020					
	Val	Val	Leu	Ser	Phe	Tyr	Asp	Glu	Thr	Ile	Lys	Gln	Met	Thr	Thr	Leu	
	1025					1030					1035					1040	
40	Gly	Tyr	Ile	Asn	Gln	Lys	Asp	Ser	Met	Phe	Asn	Asn	Phe	Ile	Gln	Ser	
				1045						1050					1055		
	Phe	Asn	Pro	Ser	Asp	Ile	Arg	Leu	Ile								
45				1060					1065								

The present invention also relates to the *S. aureus dnaX* gene. This *S. aureus dnaX* gene has a partial nucleotide sequence corresponding to SEQ. ID. No. 3 as follows:

50	ttgaattatc	aagccttata	tcgatatgtac	agaccccaaa	gtttcgagga	tgtcgctcgga	60
	caagaacatg	tcacgaagac	attgcgcaat	gcgatttcga	aagaaaaaca	gtcgcatgca	120
	tatattttta	gtgggtccgag	aggtacgggg	aaaacgagta	ttgccaaagt	gtttgctaaa	180
	gcaatcaact	gtttaaatag	cactgatgga	gaaccttgta	atgaatgtca	tatttgtaaa	240
	ggcattacgc	aggggactaa	ttcagatgtg	atagaaattg	atgctgctag	taataatggc	300
55	gttgatgaaa	taagaaatat	tagagacaaa	gttaaataatg	caccaagtga	atcgaaatat	360
	aaagtttata	ttatagatga	ggtgcacatg	ctaacaacag	gtgcttttta	tgccctttta	420
	aagacgttag	aagaacctcc	agcacacgct	atttttatat	tggcaacgac	agaaccacat	480
	aaaatccctc	caacaatcat	ttctagggca	caacgttttg	attttaaagc	aattagccta	540
	gatcaaatg	ttgaacgttt	aaaatttgta	gcagatgcac	aacaaattga	atgtgaagat	600
60	gaagccttgg	catttatcgc	taaagcgtct	gaaggggggta	tgcgtgatgc	attaagtatt	660

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	atggatcagg	ctattgcttt	cggcgatggc	acattgacat	tacaagatgc	cctaaatggt	720
	acgggtagcg	ttcatgatga	agcgttggat	cacttgtttg	atgatattgt	acaagggtgac	780
	gtacaagcat	cttttaaaaa	ataccatcag	tttataacag	aaggtaaaga	agtgaatcgc	840
5	ctaataaatg	atatgattta	ttttgtcaga	gatacgatta	tgaataaaac	atctgagaaa	900
	gatactgagt	atcgagcact	gatgaactta	gaattagata	tgttatatca	aatgattgat	960
	cttattaatg	atacattagt	gtcgattcgt	tttagtgtga	atcaaaacgt	tcatttttgaa	1020
	gtattgttag	taaaattagc	tgagcagatt	aagggtcaac	cacaagtgat	tgcgaaatgta	1080
	gctgaaccag	cacaaattgc	ttcatcgcca	aacacagatg	tattgttgca	acgtatggaa	1140
10	cagttagagc	aagaactaaa	aacactaaaa	gcacaaggag	tgagtgttgc	tcctactcaa	1200
	aaatcttcga	aaaagcctgc	gagaggtata	caaaaatcta	aaaatgcatt	ttcaatgcaa	1260
	caaattgcaa	aagtgtctaga	taaagcgaat	aaggcagata	tcaaattggt	gaaagatcat	1320
	tggaagaag	tgattgacca	tgcccaaaac	aatgataaaa	aatcactcgt	tagttttattg	1380
	caaaattcgg	aacctgtggc	ggcaagtga	gatcacgtcc	ttgtgaaatt	tgaggaagag	1440
15	atccattgtg	aaatcgta	taaagacga	gagaaacgta	gtagtataga	aagtgttgta	1500
	tgtaatatcg	ttaataaaaa	cgtaaaggt	gttggtgtac	catcagatca	atggcaaaga	1560
	gttcgaacgg	agtattttaca	aaatcgtaaa	aacgaaggcg	atgatatgcc	aaagcaacaa	1620
	gcacaacaaa	cagatattgc	tcaaaaagca	aaagatcttt	tcggtgaaga	aactgtacat	1680
	gtgatagatg	aagagtga					1698

20

The *S. aureus dnaX* encoded protein (i.e., the tau subunit) has a partial amino acid sequence corresponding to SEQ. ID. No. 4 as follows:

25	Leu	Asn	Tyr	Gln	Ala	Leu	Tyr	Arg	Met	Tyr	Arg	Pro	Gln	Ser	Phe	Glu
	1				5					10					15	
	Asp	Val	Val	Gly	Gln	Glu	His	Val	Thr	Lys	Thr	Leu	Arg	Asn	Ala	Ile
				20					25					30		
30	Ser	Lys	Glu	Lys	Gln	Ser	His	Ala	Tyr	Ile	Phe	Ser	Gly	Pro	Arg	Gly
			35					40					45			
	Thr	Gly	Lys	Thr	Ser	Ile	Ala	Lys	Val	Phe	Ala	Lys	Ala	Ile	Asn	Cys
35		50					55					60				
	Leu	Asn	Ser	Thr	Asp	Gly	Glu	Pro	Cys	Asn	Glu	Cys	His	Ile	Cys	Lys
	65					70					75					80
40	Gly	Ile	Thr	Gln	Gly	Thr	Asn	Ser	Asp	Val	Ile	Glu	Ile	Asp	Ala	Ala
				85						90					95	
	Ser	Asn	Asn	Gly	Val	Asp	Glu	Ile	Arg	Asn	Ile	Arg	Asp	Lys	Val	Lys
				100					105					110		
45	Tyr	Ala	Pro	Ser	Glu	Ser	Lys	Tyr	Lys	Val	Tyr	Ile	Ile	Asp	Glu	Val
			115					120					125			
	His	Met	Leu	Thr	Thr	Gly	Ala	Phe	Asn	Ala	Leu	Leu	Lys	Thr	Leu	Glu
50		130				135						140				
	Glu	Pro	Pro	Ala	His	Ala	Ile	Phe	Ile	Leu	Ala	Thr	Thr	Glu	Pro	His
	145					150					155					160
	Lys	Ile	Pro	Pro	Thr	Ile	Ile	Ser	Arg	Ala	Gln	Arg	Phe	Asp	Phe	Lys
55					165					170				175		
	Ala	Ile	Ser	Leu	Asp	Gln	Ile	Val	Glu	Arg	Leu	Lys	Phe	Val	Ala	Asp
				180					185					190		
60	Ala	Gln	Gln	Ile	Glu	Cys	Glu	Asp	Glu	Ala	Leu	Ala	Phe	Ile	Ala	Lys
			195				200						205			

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	Ala	Ser	Glu	Gly	Gly	Met	Arg	Asp	Ala	Leu	Ser	Ile	Met	Asp	Gln	Ala
	210						215					220				
5	Ile	Ala	Phe	Gly	Asp	Gly	Thr	Leu	Thr	Leu	Gln	Asp	Ala	Leu	Asn	Val
	225					230					235					240
	Thr	Gly	Ser	Val	His	Asp	Glu	Ala	Leu	Asp	His	Leu	Phe	Asp	Asp	Ile
10					245					250					255	
	Val	Gln	Gly	Asp	Val	Gln	Ala	Ser	Phe	Lys	Lys	Tyr	His	Gln	Phe	Ile
				260					265					270		
15	Thr	Glu	Gly	Lys	Glu	Val	Asn	Arg	Leu	Ile	Asn	Asp	Met	Ile	Tyr	Phe
			275					280					285			
	Val	Arg	Asp	Thr	Ile	Met	Asn	Lys	Thr	Ser	Glu	Lys	Asp	Thr	Glu	Tyr
	290						295					300				
20	Arg	Ala	Leu	Met	Asn	Leu	Glu	Leu	Asp	Met	Leu	Tyr	Gln	Met	Ile	Asp
	305					310					315					320
	Leu	Ile	Asn	Asp	Thr	Leu	Val	Ser	Ile	Arg	Phe	Ser	Val	Asn	Gln	Asn
25					325					330					335	
	Val	His	Phe	Glu	Val	Leu	Leu	Val	Lys	Leu	Ala	Glu	Gln	Ile	Lys	Gly
				340					345					350		
30	Gln	Pro	Gln	Val	Ile	Ala	Asn	Val	Ala	Glu	Pro	Ala	Gln	Ile	Ala	Ser
			355					360					365			
	Ser	Pro	Asn	Thr	Asp	Val	Leu	Leu	Gln	Arg	Met	Glu	Gln	Leu	Glu	Gln
	370						375					380				
35	Glu	Leu	Lys	Thr	Leu	Lys	Ala	Gln	Gly	Val	Ser	Val	Ala	Pro	Thr	Gln
	385					390					395					400
	Lys	Ser	Ser	Lys	Lys	Pro	Ala	Arg	Gly	Ile	Gln	Lys	Ser	Lys	Asn	Ala
40					405					410					415	
	Phe	Ser	Met	Gln	Gln	Ile	Ala	Lys	Val	Leu	Asp	Lys	Ala	Asn	Lys	Ala
				420					425					430		
45	Asp	Ile	Lys	Leu	Leu	Lys	Asp	His	Trp	Gln	Glu	Val	Ile	Asp	His	Ala
			435					440					445			
	Gln	Asn	Asn	Asp	Lys	Lys	Ser	Leu	Val	Ser	Leu	Leu	Gln	Asn	Ser	Glu
	450						455					460				
50	Pro	Val	Ala	Ala	Ser	Glu	Asp	His	Val	Leu	Val	Lys	Phe	Glu	Glu	Glu
	465					470					475					480
	Ile	His	Cys	Glu	Ile	Val	Asn	Lys	Asp	Asp	Glu	Lys	Arg	Ser	Ser	Ile
55					485					490					495	
	Glu	Ser	Val	Val	Cys	Asn	Ile	Val	Asn	Lys	Asn	Val	Lys	Val	Val	Gly
				500					505					510		
60	Val	Pro	Ser	Asp	Gln	Trp	Gln	Arg	Val	Arg	Thr	Glu	Tyr	Leu	Gln	Asn
			515					520					525			
	Arg	Lys	Asn	Glu	Gly	Asp	Asp	Met	Pro	Lys	Gln	Gln	Ala	Gln	Gln	Thr
	530						535					540				

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Asp Ile Ala Gln Lys Ala Lys Asp Leu Phe Gly Glu Glu Thr Val His
545 550 555 560

Val Ile Asp Glu Glu Glx
565

5

The tau subunit of *S. aureus* functions as does both the tau subunit and the gamma subunit of *E. coli*.

This invention also relates to the partial nucleotide sequence of the *S. aureus dnaB* gene. The partial nucleotide sequence of this *dnaB* gene corresponds to SEQ. ID. No. 5 as follows:

10

atggatagaa tgtatgagca aaatcaaattg ccgcataaca atgaagctga acagtctgtc 60
ttaggttcaa ttattataga tccagaattg attaataacta ctcaggaagt tttgcttcct 120
15 gagtcgtttt ataggggtgc ccatcaacat attttccgtg caatgatgca cttaaataagaa 180
gataataaag aaattgatgt tgtaacattg atggatcaat tatcgacgga aggtacgttg 240
aatgaagcgg gtggcccgca atatcttgca gagttatcta caaatgtacc aacgacgcga 300
aatgttcagt attatactga tatcgtttct aagcatgcat taaaacgtag attgattcaa 360
actgcagata gtattgccaa tgatggatat aatgatgaac ttgaactaga tgcgatttta 420
20 agtgatgcag aacgtcgaat tttagagcta tcatcttctc gtgaaagcga tggcttttaa 480
gacattcgag acgtcttagg acaagtgtat gaaacagctg aagagcttga tcaaaatagt 540
ggtcaaacac caggtatacc tacaggatat cgagatttag accaaatgac agcaggggttc 600
aacggaaatg atttaattat ccttgccagcg cgtccatctg taggtaagac tgcgttcgca 660
cttaatatgg cacaaaaagt tgcaacgcac gaagatatgt atacagttgg tattttctcg 720
25 ctagagatgg gtgctgatca gttagccaca cgtatgattt gtagttctgg aaatgttgac 780
tcaaaccgct taagaacggg tactatgact gaggaagatt ggagtcgttt tactatagcg 840
gtaggtaaat tatcacgtac gaagattttt attgatgata caccgggtat tccaattaat 900
gatttacgtt ctaaattgtcg tcgattaaag caagaacatg gcttagacat gatttgtgatt 960
gactacttac agttgattca aggtagtggg tcacgtgctg ccgataacag acaacaggaa 1020
30 gtttctgaaa tctctcgtac attaaaagca ttagcccggtg aattaaaatg tccagttatc 1080
gcattaagtc agttatctcg tgggtgttgaa caacgacaag ataaacgtcc aatgatgagt 1140
gatattcgtg aatctgggtc gattgagcaa gatgccgata tcggttcatt cttataccgt 1200
gatgattact ataaccgtgg cggcgatgaa cgtatgacg atgatggtgg tttcgagcca 1260
caaacgaatg atgaaaacgg tgaaattgaa attatcattg ctaagcaacg taacgggtcca 1320
35 acaggcacag ttaagttaca ttttatgaaa caatataata aatttaccga tatcgattat 1380
gcacatgcag atatgatg 1398

The amino acid sequence of *S. aureus* DnaB encoded by the *dnaB* gene corresponds to SEQ. ID. No. 6 as follows:

40

Met Asp Arg Met Tyr Glu Gln Asn Gln Met Pro His Asn Asn Glu Ala
1 5 10 15
45 Glu Gln Ser Val Leu Gly Ser Ile Ile Ile Asp Pro Glu Leu Ile Asn
20 25 30
Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His
35 40 45
50 Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu
50 55 60
Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu
65 70 75 80

55

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	Asn	Glu	Ala	Gly	Gly	Pro	Gln	Tyr	Leu	Ala	Glu	Leu	Ser	Thr	Asn	Val	
					85					90					95		
5	Pro	Thr	Thr	Arg	Asn	Val	Gln	Tyr	Tyr	Thr	Asp	Ile	Val	Ser	Lys	His	
				100					105					110			
	Ala	Leu	Lys	Arg	Arg	Leu	Ile	Gln	Thr	Ala	Asp	Ser	Ile	Ala	Asn	Asp	
			115					120					125				
10	Gly	Tyr	Asn	Asp	Glu	Leu	Glu	Leu	Asp	Ala	Ile	Leu	Ser	Asp	Ala	Glu	
		130					135					140					
	Arg	Arg	Ile	Leu	Glu	Leu	Ser	Ser	Ser	Arg	Glu	Ser	Asp	Gly	Phe	Lys	
15		145				150					155					160	
	Asp	Ile	Arg	Asp	Val	Leu	Gly	Gln	Val	Tyr	Glu	Thr	Ala	Glu	Glu	Leu	
					165					170					175		
20	Asp	Gln	Asn	Ser	Gly	Gln	Thr	Pro	Gly	Ile	Pro	Thr	Gly	Tyr	Arg	Asp	
				180					185					190			
	Leu	Asp	Gln	Met	Thr	Ala	Gly	Phe	Asn	Arg	Asn	Asp	Leu	Ile	Ile	Leu	
			195					200					205				
25	Ala	Ala	Arg	Pro	Ser	Val	Gly	Lys	Thr	Ala	Phe	Ala	Leu	Asn	Ile	Ala	
		210					215					220					
	Gln	Lys	Val	Ala	Thr	His	Glu	Asp	Met	Tyr	Thr	Val	Gly	Ile	Phe	Ser	
30		225				230					235					240	
	Leu	Glu	Met	Gly	Ala	Asp	Gln	Leu	Ala	Thr	Arg	Met	Ile	Cys	Ser	Ser	
				245						250				255			
35	Gly	Asn	Val	Asp	Ser	Asn	Arg	Leu	Arg	Thr	Gly	Thr	Met	Thr	Glu	Glu	
			260						265					270			
	Asp	Trp	Ser	Arg	Phe	Thr	Ile	Ala	Val	Gly	Lys	Leu	Ser	Arg	Thr	Lys	
			275					280					285				
40	Ile	Phe	Ile	Asp	Asp	Thr	Pro	Gly	Ile	Arg	Ile	Asn	Asp	Leu	Arg	Ser	
		290					295					300					
	Lys	Cys	Arg	Arg	Leu	Lys	Gln	Glu	His	Gly	Leu	Asp	Met	Ile	Val	Ile	
45		305				310					315					320	
	Asp	Tyr	Leu	Gln	Leu	Ile	Gln	Gly	Ser	Gly	Ser	Arg	Ala	Ser	Asp	Asn	
				325						330					335		
50	Arg	Gln	Gln	Glu	Val	Ser	Glu	Ile	Ser	Arg	Thr	Leu	Lys	Ala	Leu	Ala	
				340					345					350			
	Arg	Glu	Leu	Lys	Cys	Pro	Val	Ile	Ala	Leu	Ser	Gln	Leu	Ser	Arg	Gly	
			355					360					365				
55	Val	Glu	Gln	Arg	Gln	Asp	Lys	Arg	Pro	Met	Met	Ser	Asp	Ile	Arg	Glu	
		370				375						380					
	Ser	Gly	Ser	Ile	Glu	Gln	Asp	Ala	Asp	Ile	Val	Ala	Phe	Leu	Tyr	Arg	
60		385				390					395					400	
	Asp	Asp	Tyr	Tyr	Asn	Arg	Gly	Gly	Asp	Glu	Asp	Asp	Asp	Asp	Asp	Gly	
					405					410					415		

atgacagagc	aacaaaaaatt	taaagtgctt	gctgatcaaa	ttaaaatttc	aatcaatta	60
gatgctgaaa	ttttaaaattc	aggtgaactg	acacgtatag	atgtttctaa	caaaaacaga	120
acatgggaaat	ttcatattac	attaccacaa	ttcttagctc	atgaagatta	tttattattt	180
ataaatgcaa	tagagcaaga	gtttaaagat	atcgccaacg	ttcagatgctg	ttttacggtg	240
acaaatggca	cgaatcaaga	tgaactatgca	attaaatact	ttgggcactg	tattgaccaa	300
acagctttat	ctccaaaagt	taaaggtcaa	ttgaaacaga	aaaagcttat	tatgtctgga	360
aaagtattaa	aagtaatggt	atcaaatgac	attgaacgta	atcatttttg	taaggcatgt	420
aatggaagtc	ttatcaaagc	gttttagaagt	tgtggttttg	atatacgataa	aatcatattc	480
gaaacaaatg	ataatgatca	agaacaaaac	ttagctttct	tagaagcaca	tattcaagaa	540
gaagacgaac	aaagtgacgc	attggcaaca	gagaaacttg	aaaaaatgaa	agctgaaaaa	600
gcgaacaac	aagataacaa	cgaaagtgtc	gtcgataagt	gtcaaattgg	taagccgatt	660
caaattgaaa	atattaaacc	aattgaaatc	attattgagg	aagagtttaa	agttgcaata	720
gaggggtgtc	tttttgatat	aaacttaaaa	gaacttaaaa	gtgtgcgcca	tctcgtagaa	780
atataaagtga	ctgactatac	ggactcttta	gttttaaaaa	tgtttactcg	taaaaacaaa	840
gatgatttag	aacattttta	agcgctaagt	gttggtaaat	gggttagggc	tcaaggtcgt	900
attgaagaag	atacatttat	tagagattta	gttatgatga	tgtctgatat	tgaagagatt	960
aaaaaaagcg	caaaaaaaga	taaggctgaa	gaaaagcgtg	tagaattcca	cttgcatact	1020
gcaatggagc	aaatggatgt	tatacccaat	attggtgcgt	atgttaacaa	ggcagcagac	1080
tggggacatc	cagccattgc	ggttacagac	cataattgtg	tgcaagcatt	tccagatgct	1140
cacgcagcag	cggaaaaaca	tggcattaaa	atgatatacg	gtatggaagg	tatgttagtt	1200
gatgatggtg	ttccgatgtc	atacaaacca	caagatgtcg	tattaaaaga	tgctacttat	1260
gttggtgttc	acggttgagac	aactggttta	tcaaatcagt	atgataaaat	catcgagctt	1320
cgctgtgtga	aagttcataa	cggtgaaatc	atcgataagt	ttgaaaggtt	tagtaatccg	1380
catgaacgat	tatcggaac	gattatcaat	ttgacgcata	ttactgatga	tatgttagta	1440
gatgcccctg	agattgaaga	agtaactaca	gagtttaaa	aatgggttgg	cgatgcgata	1500
ttcgttaggc	ataatgcttc	gtttgatatg	ggcttcatcg	atacgggata	tgaacgtctt	1560
gggtttggac	catcaacgaa	tgggtgtatc	gatactttag	aatatctctg	tacgattaat	1620
actgaatatg	gtaaacatgg	tttgaatttc	ttggctaaaa	aatatggcgt	agaattaacg	1680
caacatcacc	gtgccattta	tgatacagaa	gcaacagctt	acattttcat	aaaaattggt	1740
caacaaatga	aagaattagg	cgtattaaat	cataacgaaa	tcaacaaaaa	actcagtaat	1800
gaagatgcgt	ataaacgtgc	agacactagt	catgtcacat	taattgtaca	aaaccaacaa	1860
ggctcttaaaa	atctatttta	aattgtaagt	gcatcattgg	tgaagtattt	ctaccgtaca	1920
cctcgaattc	cacgttcatt	gttagatgaa	tatcgtgagg	gattattggt	aggtacagcg	1980
tgtgatgaag	gtgaattatt	tacggcagtt	atgcagaagg	accagagtca	agttgaaaaa	2040
attggcaaat	attatgatct	tattgaaatt	caaccacccg	cactttatca	agatttaatt	2100
gatagagagc	ttattagaga	tactgaaaca	ttacatgaaa	tttatcaacg	tttaatacat	2160
gcaggtgaca	cagcgggtat	acctgttatt	gcgacaggaa	atgcacacta	tttgtttgaa	2220
catgatggta	tcgcacgtaa	aattttaata	gcatcacaac	ccggcaatcc	acttaatcgc	2280
tcaactttac	cggaagcaca	ttttagaact	acagatgaaa	tgttaaacga	gtttcatttt	2340
ttaggtgaa	aaaaagcgca	tgaatttggt	gtgaaaaata	taaacgaatt	agcagatcga	2400
attgaaagtc	ttgttcctat	taaagatgaa	ttatacacac	cgcgtatgga	aggtgctaac	2460
gaagaaatta	gagaactaag	ttatgcaaat	gcgcgtaaac	tgtatggtga	agacctgcct	2520
caaatcgtaa	ttgatcgatt	agaaaaagaa	ttaaaaagta	ttatcggtaa	tggatttgcg	2580
gtaattttact	taatttcgca	acgttttagtt	aaaaaatcat	tagatgatgg	atacttagtt	2640
gggtcccggtg	gttcagtagg	ttctagtttt	gtacgcacaa	tgaactgatg	tactgaagta	2700

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5 aaccggttac cgccacacta tatttgtccg aactgtaaaa cgagtgaatt tttcaatgat 2760
 gggtcagtag gatcaggatt tgatttacct gataagacgt gtgaaacttg tggagcgcca 2820
 cttattaaag aaggacaaga tattccggtt gaaacatttt taggatttaa gggagataaa 2880
 gttcctgata tcgacttaaa ctttagtggt gaatatcaac cgaatgccca taactacaca 2940
 aaagtattat ttggtgagga taaagtattc cgtgcaggta caattggtac tgttgctgaa 3000
 aagactgctt ttggttatgt taaaggttat ttgaatgata aaggtatcca caaaagaggt 3060
 gctgaaatag atcgactcgt taaaggatgt acagggttta aacgtacaac tggacagcat 3120
 ccagggggta ttattgtagt acctgattac atggatattt atgattttac gccgatacaa 3180
 10 tatcctgccg atgatcaaaa ttcagcatgg atgacgacac attttgattt ccattctatt 3240
 catgataatg tattaanaact tgatataact ggacacgatg atccaacaat gattcgtatg 3300
 cttcaagatt tatcaggaat tgatccaaaa acaataacct tagatgataa agaagttatg 3360
 cagatattta gtacacctga aagtttgggt gttactgaag atgaaatttt atgtaaaaca 3420
 ggtacatttg ggggtaccaga attcgggtaca ggattcgtgc gtcaaagtgt agaagataca 3480
 aagccaacaa cattttctga attagttcaa atctcaggat tatctcatgg tacagatgtg 3540
 15 tggtaggca atgctcaaga attaattaaa accgggtatat gtgattttatc aagtgtaat 3600
 gggtgtcgtg atgatatcat ggtttattta gtgtatgctg gtttagaacc atcaatggct 3660
 tttaaaataa tggagtcagt acgtaaaggt aaagggtttaa ctgaagaaat gattgaaacg 3720
 atgaaagaaa atgaagtgcc agattggtat ttagattcat gtcttaaaat taagtacatg 3780
 20 ttccctaaag cccatgcagc agcatacggt ttaatggcag tacgtatcgc atatttcaaa 3840
 gtacatcatc cactttatta ctatgcacat tactttacaa ttcgtgcgtc agactttgat 3900
 ttaatcacga tgattaaaaga taaaacaagc attcgaaata ctgtaaaaga catgtattct 3960
 cgctatatgg atctaggtaa aaaagaaaaa gacgtattaa cagtcttgga aattatgaat 4020
 gaaatggcgc atcgagggtta tcgaatgcaa ccgattagtt tagaaaagag tcaggcggtc 4080
 25 gaatttatca ttgaaggcga tacacttatt ccgcccgttca tatcagtgcc tgggcttgcc 4140
 gaaaacgttg cgaaacgaat ttgtgaagct cgtgacgatg gcccaatttt atcaaaaaga 4200
 gatttaaaaca aaaaagctgg attatctcag aaaattattg agtatttaga tgagttaggc 4260
 tcattaccga atttaccaga taaagctcaa ctttcgatat ttgatatg 4308

30 The amino acid sequence of the *S. aureus polC* gene product, α -large,
 corresponds to SEQ. ID. No. 8 as follows:

Met Thr Glu Gln Gln Lys Phe Lys Val Leu Ala Asp Gln Ile Lys Ile
 1 5 10 15
 35 Ser Asn Gln Leu Asp Ala Glu Ile Leu Asn Ser Gly Glu Leu Thr Arg
 20 25 30
 Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
 35 40 45
 Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
 50 55 60
 45 Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
 65 70 75 80
 Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His
 85 90 95
 50 Cys Ile Asp Gln Thr Ala Leu Ser Pro Lys Val Lys Gly Gln Leu Lys
 100 105 110
 Gln Lys Lys Leu Ile Met Ser Gly Lys Val Leu Lys Val Met Val Ser
 115 120 125
 55 Asn Asp Ile Glu Arg Asn His Phe Asp Lys Ala Cys Asn Gly Ser Leu
 130 135 140
 60 Ile Lys Ala Phe Arg Asn Cys Gly Phe Asp Ile Asp Lys Ile Ile Phe
 145 150 155 160

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	Glu	Thr	Asn	Asp	Asn	Asp	Gln	Glu	Gln	Asn	Leu	Ala	Ser	Leu	Glu	Ala	
					165					170					175		
5	His	Ile	Gln	Glu	Glu	Asp	Glu	Gln	Ser	Ala	Arg	Leu	Ala	Thr	Glu	Lys	
				180					185					190			
	Leu	Glu	Lys	Met	Lys	Ala	Glu	Lys	Ala	Lys	Gln	Gln	Asp	Asn	Lys	Gln	
			195					200					205				
10	Ser	Ala	Val	Asp	Lys	Cys	Gln	Ile	Gly	Lys	Pro	Ile	Gln	Ile	Glu	Asn	
		210					215						220				
	Ile	Lys	Pro	Ile	Glu	Ser	Ile	Ile	Glu	Glu	Glu	Phe	Lys	Val	Ala	Ile	
15		225				230					235					240	
	Glu	Gly	Val	Ile	Phe	Asp	Ile	Asn	Leu	Lys	Glu	Leu	Lys	Ser	Gly	Arg	
					245					250					255		
20	His	Ile	Val	Glu	Ile	Lys	Val	Thr	Asp	Tyr	Thr	Asp	Ser	Leu	Val	Leu	
				260					265					270			
	Lys	Met	Phe	Thr	Arg	Lys	Asn	Lys	Asp	Asp	Leu	Glu	His	Phe	Lys	Ala	
			275					280					285				
25	Leu	Ser	Val	Gly	Lys	Trp	Val	Arg	Ala	Gln	Gly	Arg	Ile	Glu	Glu	Asp	
		290					295					300					
	Thr	Phe	Ile	Arg	Asp	Leu	Val	Met	Met	Met	Ser	Asp	Ile	Glu	Glu	Ile	
30		305				310					315					320	
	Lys	Lys	Ala	Thr	Lys	Lys	Asp	Lys	Ala	Glu	Glu	Lys	Arg	Val	Glu	Phe	
					325					330					335		
35	His	Leu	His	Thr	Ala	Met	Ser	Gln	Met	Asp	Gly	Ile	Pro	Asn	Ile	Gly	
				340					345					350			
	Ala	Tyr	Val	Lys	Gln	Ala	Ala	Asp	Trp	Gly	His	Pro	Ala	Ile	Ala	Val	
			355					360					365				
40	Thr	Asp	His	Asn	Val	Val	Gln	Ala	Phe	Pro	Asp	Ala	His	Ala	Ala	Ala	
		370					375					380					
	Glu	Lys	His	Gly	Ile	Lys	Met	Ile	Tyr	Gly	Met	Glu	Gly	Met	Leu	Val	
45		385				390					395					400	
	Asp	Asp	Gly	Val	Pro	Ile	Ala	Tyr	Lys	Pro	Gln	Asp	Val	Val	Leu	Lys	
					405					410					415		
50	Asp	Ala	Thr	Tyr	Val	Val	Phe	Asp	Val	Glu	Thr	Thr	Gly	Leu	Ser	Asn	
				420					425					430			
	Gln	Tyr	Asp	Lys	Ile	Ile	Glu	Leu	Ala	Ala	Val	Lys	Val	His	Asn	Gly	
			435				440						445				
55	Glu	Ile	Ile	Asp	Lys	Phe	Glu	Arg	Phe	Ser	Asn	Pro	His	Glu	Arg	Leu	
		450					455					460					
	Ser	Glu	Thr	Ile	Ile	Asn	Leu	Thr	His	Ile	Thr	Asp	Asp	Met	Leu	Val	
		465				470					475				480		
60	Asp	Ala	Pro	Glu	Ile	Glu	Glu	Val	Leu	Thr	Glu	Phe	Lys	Glu	Trp	Val	
					485					490					495		

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	Gly	Asp	Ala	Ile	Phe	Val	Ala	His	Asn	Ala	Ser	Phe	Asp	Met	Gly	Phe	
				500					505					510			
5	Ile	Asp	Thr	Gly	Tyr	Glu	Arg	Leu	Gly	Phe	Gly	Pro	Ser	Thr	Asn	Gly	
			515					520					525				
	Val	Ile	Asp	Thr	Leu	Glu	Leu	Ser	Arg	Thr	Ile	Asn	Thr	Glu	Tyr	Gly	
			530				535					540					
10	Lys	His	Gly	Leu	Asn	Phe	Leu	Ala	Lys	Lys	Tyr	Gly	Val	Glu	Leu	Thr	
	545					550					555					560	
	Gln	His	His	Arg	Ala	Ile	Tyr	Asp	Thr	Glu	Ala	Thr	Ala	Tyr	Ile	Phe	
					565					570					575		
15	Ile	Lys	Met	Val	Gln	Gln	Met	Lys	Glu	Leu	Gly	Val	Leu	Asn	His	Asn	
				580					585					590			
20	Glu	Ile	Asn	Lys	Lys	Leu	Ser	Asn	Glu	Asp	Ala	Tyr	Lys	Arg	Ala	Arg	
			595					600					605				
	Pro	Ser	His	Val	Thr	Leu	Ile	Val	Gln	Asn	Gln	Gln	Gly	Leu	Lys	Asn	
			610				615					620					
25	Leu	Phe	Lys	Ile	Val	Ser	Ala	Ser	Leu	Val	Lys	Tyr	Phe	Tyr	Arg	Thr	
	625					630					635					640	
	Pro	Arg	Ile	Pro	Arg	Ser	Leu	Leu	Asp	Glu	Tyr	Arg	Glu	Gly	Leu	Leu	
					645					650					655		
30	Val	Gly	Thr	Ala	Cys	Asp	Glu	Gly	Glu	Leu	Phe	Thr	Ala	Val	Met	Gln	
				660					665					670			
	Lys	Asp	Gln	Ser	Gln	Val	Glu	Lys	Ile	Ala	Lys	Tyr	Tyr	Asp	Phe	Ile	
			675					680					685				
35	Glu	Ile	Gln	Pro	Pro	Ala	Leu	Tyr	Gln	Asp	Leu	Ile	Asp	Arg	Glu	Leu	
		690					695					700					
40	Ile	Arg	Asp	Thr	Glu	Thr	Leu	His	Glu	Ile	Tyr	Gln	Arg	Leu	Ile	His	
	705					710					715					720	
	Ala	Gly	Asp	Thr	Ala	Gly	Ile	Pro	Val	Ile	Ala	Thr	Gly	Asn	Ala	His	
					725					730					735		
45	Tyr	Leu	Phe	Glu	His	Asp	Gly	Ile	Ala	Arg	Lys	Ile	Leu	Ile	Ala	Ser	
				740					745					750			
	Gln	Pro	Gly	Asn	Pro	Leu	Asn	Arg	Ser	Thr	Leu	Pro	Glu	Ala	His	Phe	
			755					760					765				
50	Arg	Thr	Thr	Asp	Glu	Met	Leu	Asn	Glu	Phe	His	Phe	Leu	Gly	Glu	Glu	
		770				775						780					
55	Lys	Ala	His	Glu	Ile	Val	Val	Lys	Asn	Thr	Asn	Glu	Leu	Ala	Asp	Arg	
	785					790					795					800	
	Ile	Glu	Arg	Val	Val	Pro	Ile	Lys	Asp	Glu	Leu	Tyr	Thr	Pro	Arg	Met	
					805					810					815		
60	Glu	Gly	Ala	Asn	Glu	Glu	Ile	Arg	Glu	Leu	Ser	Tyr	Ala	Asn	Ala	Arg	
				820					825					830			

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	Lys	Leu	Tyr	Gly	Glu	Asp	Leu	Pro	Gln	Ile	Val	Ile	Asp	Arg	Leu	Glu	
			835					840					845				
5	Lys	Glu	Leu	Lys	Ser	Ile	Ile	Gly	Asn	Gly	Phe	Ala	Val	Ile	Tyr	Leu	
		850					855					860					
	Ile	Ser	Gln	Arg	Leu	Val	Lys	Lys	Ser	Leu	Asp	Asp	Gly	Tyr	Leu	Val	
	865					870					875					880	
10	Gly	Ser	Arg	Gly	Ser	Val	Gly	Ser	Ser	Phe	Val	Ala	Thr	Met	Thr	Glu	
					885					890					895		
	Ile	Thr	Glu	Val	Asn	Pro	Leu	Pro	Pro	His	Tyr	Ile	Cys	Pro	Asn	Cys	
15				900					905					910			
	Lys	Thr	Ser	Glu	Phe	Phe	Asn	Asp	Gly	Ser	Val	Gly	Ser	Gly	Phe	Asp	
			915					920					925				
20	Leu	Pro	Asp	Lys	Thr	Cys	Glu	Thr	Cys	Gly	Ala	Pro	Leu	Ile	Lys	Glu	
		930					935					940					
	Gly	Gln	Asp	Ile	Pro	Phe	Glu	Lys	Phe	Leu	Gly	Phe	Lys	Gly	Asp	Lys	
	945					950					955					960	
25	Val	Pro	Asp	Ile	Asp	Leu	Asn	Phe	Ser	Gly	Glu	Tyr	Gln	Pro	Asn	Ala	
					965					970					975		
	His	Asn	Tyr	Thr	Lys	Val	Leu	Phe	Gly	Glu	Asp	Lys	Val	Phe	Arg	Ala	
30				980					985					990			
	Gly	Thr	Ile	Gly	Thr	Val	Ala	Glu	Lys	Thr	Ala	Phe	Gly	Tyr	Val	Lys	
			995					1000					1005				
35	Gly	Tyr	Leu	Asn	Asp	Gln	Gly	Ile	His	Lys	Arg	Gly	Ala	Glu	Ile	Asp	
		1010					1015					1020					
	Arg	Leu	Val	Lys	Gly	Cys	Thr	Gly	Val	Lys	Ala	Thr	Thr	Gly	Gln	His	
	1025					1030					1035					1040	
40	Pro	Gly	Gly	Ile	Ile	Val	Val	Pro	Asp	Tyr	Met	Asp	Ile	Tyr	Asp	Phe	
					1045					1050					1055		
	Thr	Pro	Ile	Gln	Tyr	Pro	Ala	Asp	Asp	Gln	Asn	Ser	Ala	Trp	Met	Thr	
45				1060					1065					1070			
	Thr	His	Phe	Asp	Phe	His	Ser	Ile	His	Asp	Asn	Val	Leu	Lys	Leu	Asp	
			1075					1080					1085				
50	Ile	Leu	Gly	His	Asp	Asp	Pro	Thr	Met	Ile	Arg	Met	Leu	Gln	Asp	Leu	
		1090					1095					1100					
	Ser	Gly	Ile	Asp	Pro	Lys	Thr	Ile	Pro	Val	Asp	Asp	Lys	Glu	Val	Met	
	1105					1110					1115				1120		
55	Gln	Ile	Phe	Ser	Thr	Pro	Glu	Ser	Leu	Gly	Val	Thr	Glu	Asp	Glu	Ile	
					1125					1130					1135		
	Leu	Cys	Lys	Thr	Gly	Thr	Phe	Gly	Val	Pro	Asn	Ser	Asp	Arg	Ile	Arg	
60				1140				1145						1150			
	Arg	Gln	Met	Leu	Glu	Asp	Thr	Lys	Pro	Thr	Thr	Phe	Ser	Glu	Leu	Val	
		1155						1160						1165			

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Gln Ile Ser Gly Leu Ser His Gly Thr Asp Val Trp Leu Gly Asn Ala
 1170 1175 1180
 5 Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly
 1185 1190 1195 1200
 Cys Arg Asp Asp Ile Met Val Tyr Leu Met Tyr Ala Gly Leu Glu Pro
 1205 1210 1215
 10 Ser Met Ala Phe Lys Ile Met Glu Ser Val Arg Lys Gly Lys Gly Leu
 1220 1225 1230
 Thr Glu Glu Met Ile Glu Thr Met Lys Glu Asn Glu Val Pro Asp Trp
 1235 1240 1245
 15 Tyr Leu Asp Ser Cys Leu Lys Ile Lys Tyr Ile Phe Pro Lys Ala His
 1250 1255 1260
 Ala Ala Ala Tyr Val Leu Met Ala Val Arg Ile Ala Tyr Phe Lys Val
 1265 1270 1275 1280
 20 His His Pro Leu Tyr Tyr Tyr Ala Ser Tyr Phe Thr Ile Arg Ala Ser
 1285 1290 1295
 25 Asp Phe Asp Leu Ile Thr Met Ile Lys Asp Lys Thr Ser Ile Arg Asn
 1300 1305 1310
 Thr Val Lys Asp Met Tyr Ser Arg Tyr Met Asp Leu Gly Lys Lys Glu
 1315 1320 1325
 30 Lys Asp Val Leu Thr Val Leu Glu Ile Met Asn Glu Met Ala His Arg
 1330 1335 1340
 Gly Tyr Arg Met Gln Pro Ile Ser Leu Glu Lys Ser Gln Ala Phe Glu
 1345 1350 1355 1360
 35 Phe Ile Ile Glu Gly Asp Thr Leu Ile Pro Pro Phe Ile Ser Val Pro
 1365 1370 1375
 40 Gly Leu Gly Glu Asn Val Ala Lys Arg Ile Val Glu Ala Arg Asp Asp
 1380 1385 1390
 Gly Pro Phe Leu Ser Lys Glu Asp Leu Asn Lys Lys Ala Gly Leu Tyr
 1395 1400 1405
 45 Gln Lys Ile Ile Glu Tyr Leu Asp Glu Leu Gly Ser Leu Pro Asn Leu
 1410 1415 1420
 50 Pro Asp Lys Ala Gln Leu Ser Ile Phe Asp Met
 1425 1430 1435

This invention also relates to the *S. aureus dnaN* gene encoding the
 beta subunit. The partial nucleotide sequence of this *dnaN* gene corresponds to SEQ.
 ID. No. 9 as follows:

55

atgatggaat	tcactattaa	aagagattat	tttattacac	aattaaatga	cacattaaaa	60
gctatttcac	caagaacaac	attacctata	ttactggta	tcaaaatcga	tgcgaaagaa	120
catgaagtta	tattaactgg	ttcagactct	gaaatttcaa	tagaaatcac	tattcctaaa	180
actgtagatg	gcgaagatat	tgtcaatatt	tcagaaacag	gctcagtagt	acttcctgga	240

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	cgattctttg	ttgatattat	aaaaaaatta	cctggtaaag	atgttaaatt	atctacaaat	300
	gaacaattcc	agacattaat	tacatcaggt	cattctgaat	ttaatttgag	tggttagat	360
	ccagatcaat	atcctttatt	acctcaagtt	tctagagatg	acgcaattca	attgtcggt	420
5	aaagtactta	aaaacgtgat	tgcacaaacg	aattttgcag	tgtccacctc	agaaacacgc	480
	ccagtactaa	ctggtgtgaa	ctggcttata	caagaaaatg	aattaatatg	cacagcgact	540
	gattcacacc	gcttggctgt	aagaaaagttg	cagttagaag	atgtttctga	aaacaaaaat	600
	gtcatcattc	caggtaaggc	tttagctgaa	ttaaataaaa	ttatgtctga	caatgaagaa	660
	gacattgata	tcttctttgc	ttcaaaccac	gttttattta	aagttggaaa	tgtgaacttt	720
10	atctctcgat	tattagaagg	acattatcct	gatacaacac	gtttattccc	tgaaaactat	780
	gaaattaaat	taagtataga	caatggggag	ttttatcatg	cgattgatcg	tgctcttta	840
	ttagcacgtg	aaggtggtaa	taacgttatt	aaattaagta	caggtgatga	cgttgttgaa	900
	ttatcttcta	catcaccaga	aattgggtact	gtaaaagaag	aagttgatgc	aaacgatgtt	960
	gaaggtggta	gcctgaaaat	ttcattcaac	tctaaatata	tgatggatgc	tttaaaagca	1020
15	atcgataatg	atgaggttga	agttgaattc	ttcgggtacaa	tgaaaccatt	tattctaaaa	1080
	cctaaaggtg	acgactcggt	aacgcaatta	attttaccaa	tcagaactta	ctaa	1134

This amino acid sequence of *S. aureus* beta subunit is as follows (SEQ.
ID. No. 10):

20	Met	Met	Glu	Phe	Thr	Ile	Lys	Arg	Asp	Tyr	Phe	Ile	Thr	Gln	Leu	Asn
	1				5					10					15	
	Asp	Thr	Leu	Lys	Ala	Ile	Ser	Pro	Arg	Thr	Thr	Leu	Pro	Ile	Leu	Thr
25				20					25					30		
	Gly	Ile	Lys	Ile	Asp	Ala	Lys	Glu	His	Glu	Val	Ile	Leu	Thr	Gly	Ser
			35					40					45			
30	Asp	Ser	Glu	Ile	Ser	Ile	Glu	Ile	Thr	Ile	Pro	Lys	Thr	Val	Asp	Gly
		50					55					60				
	Glu	Asp	Ile	Val	Asn	Ile	Ser	Glu	Thr	Gly	Ser	Val	Val	Leu	Pro	Gly
	65				70					75						80
35	Arg	Phe	Phe	Val	Asp	Ile	Ile	Lys	Lys	Leu	Pro	Gly	Lys	Asp	Val	Lys
				85						90					95	
	Leu	Ser	Thr	Asn	Glu	Gln	Phe	Gln	Thr	Leu	Ile	Thr	Ser	Gly	His	Ser
40				100					105					110		
	Glu	Phe	Asn	Leu	Ser	Gly	Leu	Asp	Pro	Asp	Gln	Tyr	Pro	Leu	Leu	Pro
			115					120					125			
45	Gln	Val	Ser	Arg	Asp	Asp	Ala	Ile	Gln	Leu	Ser	Val	Lys	Val	Leu	Lys
		130					135						140			
	Asn	Val	Ile	Ala	Gln	Thr	Asn	Phe	Ala	Val	Ser	Thr	Ser	Glu	Thr	Arg
	145					150					155					160
50	Pro	Val	Leu	Thr	Gly	Val	Asn	Trp	Leu	Ile	Gln	Glu	Asn	Glu	Leu	Ile
					165					170					175	
	Cys	Thr	Ala	Thr	Asp	Ser	His	Arg	Leu	Ala	Val	Arg	Lys	Leu	Gln	Leu
55				180					185					190		
	Glu	Asp	Val	Ser	Glu	Asn	Lys	Asn	Val	Ile	Ile	Pro	Gly	Lys	Ala	Leu
			195					200					205			
60	Ala	Glu	Leu	Asn	Lys	Ile	Met	Ser	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Ile
		210					215						220			

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	Phe	Phe	Ala	Ser	Asn	Gln	Val	Leu	Phe	Lys	Val	Gly	Asn	Val	Asn	Phe
	225					230					235					240
5	Ile	Ser	Arg	Leu	Leu	Glu	Gly	His	Tyr	Pro	Asp	Thr	Thr	Arg	Leu	Phe
				245						250					255	
	Pro	Glu	Asn	Tyr	Glu	Ile	Lys	Leu	Ser	Ile	Asp	Asn	Gly	Glu	Phe	Tyr
			260						265				270			
10	His	Ala	Ile	Asp	Arg	Ala	Ser	Leu	Leu	Ala	Arg	Glu	Gly	Gly	Asn	Asn
		275						280					285			
	Val	Ile	Lys	Leu	Ser	Thr	Gly	Asp	Asp	Val	Val	Glu	Leu	Ser	Ser	Thr
15		290					295					300				
	Ser	Pro	Glu	Ile	Gly	Thr	Val	Lys	Glu	Glu	Val	Asp	Ala	Asn	Asp	Val
	305				310						315					320
20	Glu	Gly	Gly	Ser	Leu	Lys	Ile	Ser	Phe	Asn	Ser	Lys	Tyr	Met	Met	Asp
				325						330					335	
	Ala	Leu	Lys	Ala	Ile	Asp	Asn	Asp	Glu	Val	Glu	Val	Glu	Phe	Phe	Gly
			340						345					350		
25	Thr	Met	Lys	Pro	Phe	Ile	Leu	Lys	Pro	Lys	Gly	Asp	Asp	Ser	Val	Thr
			355					360					365			
	Gln	Leu	Ile	Leu	Pro	Ile	Arg	Thr	Tyr							
30		370					375									

This invention also relates to the *S. aureus hola* gene encoding the delta subunit. The partial nucleotide sequence of this *hola* gene corresponds to SEQ. ID. No. 11 as follows:

35	atggatgaac	agcaacaatt	gacgaatgca	tatcattcaa	ataaattatc	gcatgcctat	60
	ttatttgaag	gtgatgatgc	acaaacgatg	aaacaagttg	cgattaattt	tgcaaagctt	120
	attttatgtc	aaacagatag	tcaatgtgaa	acaaaggtta	gtacatatata	tcattccagac	180
	tttatgtata	tatcaacaac	tgagaatgca	attaagaaaag	aacaagttga	acaacttgtg	240
40	cgtcatatga	atcaacttcc	tatagaaaagc	acaaataaag	tgtacatcat	cgaagacttt	300
	gaagactttg	aaaagttaac	tggttcaagg	gaaaacagta	tcttgaatt	tcttgaagaa	360
	ccaccggaca	atacgattgc	tattttattg	tctacaaaac	ctgagcaaat	tttagacaca	420
	atccattcaa	ggtgtcagca	tgtatatattc	aagcctattg	ataaagaaaa	gtttataaat	480
	agattagttg	aacaaaacat	gtctaagcca	gtagctgaaa	tgattagtac	ttatactacg	540
	caaataagata	atgcaatggc	tttaaatgaa	gaatttgatt	tattagcatt	aaggaaatca	600
45	gttatacggt	gggaattggt	gcttactaat	aagccaatgg	cacttatagg	tattattgat	660
	ttattgaaac	aggctaaaaa	taaaaaactg	caatctttta	ctattgcagc	tgtgaatggt	720
	ttcttcgaag	atatcataca	tacaaaggta	aatgtagagg	ataaacaaat	atatagtgat	780
	ttaaaaaatg	atattgatca	atatgcgcaa	aagttgtcgt	ttaatcaatt	aattttgatg	840
50	tttgatcaac	tgacggaagc	acataagaaa	ttgaatcaaa	atgtaaatcc	aacgcttgta	900
	tttgaacaaa	tcgtaattaa	gggtgtgagt				930

The amino acid sequence of the delta subunit encoded by *S. aureus hola* corresponds to SEQ. ID. No. 12 as follows:

55	Met	Asp	Glu	Gln	Gln	Gln	Leu	Thr	Asn	Ala	Tyr	His	Ser	Asn	Lys	Leu
	1				5					10					15	

	Ser	His	Ala	Tyr	Leu	Phe	Glu	Gly	Asp	Asp	Ala	Gln	Thr	Met	Lys	Gln	
				20					25					30			
5	Val	Ala	Ile	Asn	Phe	Ala	Lys	Leu	Ile	Leu	Cys	Gln	Thr	Asp	Ser	Gln	
			35					40					45				
	Cys	Glu	Thr	Lys	Val	Ser	Thr	Tyr	Asn	His	Pro	Asp	Phe	Met	Tyr	Ile	
10		50					55					60					
	Ser	Thr	Thr	Glu	Asn	Ala	Ile	Lys	Lys	Glu	Gln	Val	Glu	Gln	Leu	Val	
	65					70					75					80	
15	Arg	His	Met	Asn	Gln	Leu	Pro	Ile	Glu	Ser	Thr	Asn	Lys	Val	Tyr	Ile	
				85						90					95		
	Ile	Glu	Asp	Phe	Glu	Asp	Phe	Glu	Lys	Leu	Thr	Val	Gln	Gly	Glu	Asn	
			100						105					110			
20	Ser	Ile	Leu	Lys	Phe	Leu	Glu	Glu	Pro	Pro	Asp	Asn	Thr	Ile	Ala	Ile	
		115						120					125				
	Leu	Leu	Ser	Thr	Lys	Pro	Glu	Gln	Ile	Leu	Asp	Thr	Ile	His	Ser	Arg	
25		130					135					140					
	Cys	Gln	His	Val	Tyr	Phe	Lys	Pro	Ile	Asp	Lys	Glu	Lys	Phe	Ile	Asn	
	145					150				155						160	
30	Arg	Leu	Val	Glu	Gln	Asn	Met	Ser	Lys	Pro	Val	Ala	Glu	Met	Ile	Ser	
				165						170					175		
	Thr	Tyr	Thr	Thr	Gln	Ile	Asp	Asn	Ala	Met	Ala	Leu	Asn	Glu	Glu	Phe	
			180						185					190			
35	Asp	Leu	Leu	Ala	Leu	Arg	Lys	Ser	Val	Ile	Arg	Trp	Glu	Leu	Leu	Leu	
		195						200					205				
	Thr	Asn	Lys	Pro	Met	Ala	Leu	Ile	Gly	Ile	Ile	Asp	Leu	Leu	Lys	Gln	
40		210					215					220					
	Ala	Lys	Asn	Lys	Lys	Leu	Gln	Ser	Leu	Thr	Ile	Ala	Ala	Val	Asn	Gly	
	225					230					235					240	
45	Phe	Phe	Glu	Asp	Ile	Ile	His	Thr	Lys	Val	Asn	Val	Glu	Asp	Lys	Gln	
				245						250					255		
	Ile	Tyr	Ser	Asp	Leu	Lys	Asn	Asp	Ile	Asp	Gln	Tyr	Ala	Gln	Lys	Leu	
			260					265					270				
50	Ser	Phe	Asn	Gln	Leu	Ile	Leu	Met	Phe	Asp	Gln	Leu	Thr	Glu	Ala	His	
		275						280					285				
	Lys	Lys	Leu	Asn	Gln	Asn	Val	Asn	Pro	Thr	Leu	Val	Phe	Glu	Gln	Ile	
55		290					295					300					
	Val	Ile	Lys	Gly	Val	Ser											
	305					310											

60 This invention also relates to the *S. aureus holB* gene encoding the delta prime subunit. The partial nucleotide sequence of this *holB* gene corresponds to SEQ. ID. No. 13 as follows:

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5   atgagcgaca atattgtagc tatttatgga gatgtgcctg aattgggtga aaaacaaagt 60
    gcagaaatca tatcacaatt tttgaaaagt gatagagatg actttaactt tgtgaaatat 120
    aattttatacg aaacagagat tgcaccaatt gttgaagaaa cattaacatt gccttttctt 180
    tcagataaaa aagcaatttt gggttaaaaat gcatatatat ttacaggtga aaaagcgcca 240
    aaagatatgg ctcataatgt agaccaatta atagaattta ttgaaaaata tgatggcgaa 300
    aatttgattg tctttgagat atatcaaaat aaacttgatg aaagaaaaaa gttaactaaa 360
    actctaaaaa agcatgcaag gcttaaaaaa atagagcaga tgtcggagga gatcaagtgg 420
10  attcaaaaaa aagaacaagc gattgatttt gtaaaagatc ttataacaat gaaagaagaa 480
    ccaattaaac ttcttgcact tacatcaaat tatagacttt tttatcaatg taaaattctt 540
    tcacaaaaag gttatagtgg tcaacaaatt gcaaaaacaa taggtgttca tccatataga 600
    gtgaaacttg cacttggtca agtgagacat tatcaacttg atgaacttct taatattatt 660
    gatgcatgtg cagaaacaga ttataaactt aaatcatcat atatggataa acaacttatt 720
15  cttgaacttt ttattctttc actt                                     744

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The amino acid sequence of the delta prime subunit encoded by *S. aureus holB* corresponds to SEQ. ID. No. 14 as follows:

```

20  Met Ser Asp Asn Ile Val Ala Ile Tyr Gly Asp Val Pro Glu Leu Val
    1           5           10           15
    Glu Lys Gln Ser Ala Glu Ile Ile Ser Gln Phe Leu Lys Ser Asp Arg
           20           25           30
25  Asp Asp Phe Asn Phe Val Lys Tyr Asn Leu Tyr Glu Thr Glu Ile Ala
           35           40           45
    Pro Ile Val Glu Glu Thr Leu Thr Leu Pro Phe Phe Ser Asp Lys Lys
    50           55           60
30  Ala Ile Leu Val Lys Asn Ala Tyr Ile Phe Thr Gly Glu Lys Ala Pro
    65           70           75           80
    Lys Asp Met Ala His Asn Val Asp Gln Leu Ile Glu Phe Ile Glu Lys
    85           90           95
35  Tyr Asp Gly Glu Asn Leu Ile Val Phe Glu Ile Tyr Gln Asn Lys Leu
           100          105          110
    Asp Glu Arg Lys Lys Leu Thr Lys Thr Leu Lys Lys His Ala Arg Leu
    115          120          125
40  Lys Lys Ile Glu Gln Met Ser Glu Glu Ile Lys Trp Ile Gln Lys Lys
    130          135          140
45  Glu Gln Ala Ile Asp Phe Val Lys Asp Leu Ile Thr Met Lys Glu Glu
    145          150          155          160
    Pro Ile Lys Leu Leu Ala Leu Thr Ser Asn Tyr Arg Leu Phe Tyr Gln
    165          170          175
50  Cys Lys Ile Leu Ser Gln Lys Gly Tyr Ser Gly Gln Gln Ile Ala Lys
           180          185          190
55  Thr Ile Gly Val His Pro Tyr Arg Val Lys Leu Ala Leu Gly Gln Val
    195          200          205
    Arg His Tyr Gln Leu Asp Glu Leu Leu Asn Ile Ile Asp Ala Cys Ala
    210          215          220
60

```

Glu Thr Asp Tyr Lys Leu Lys Ser Ser Tyr Met Asp Lys Gln Leu Ile
 225 230 235 240
 5 Leu Glu Leu Phe Ile Leu Ser Leu
 245

This invention also relates to the *S. aureus dnaG* gene encoding a
 primase. The partial nucleotide sequence of this *dnaG* gene corresponds to SEQ. ID.
 No. 15 as follows:

10
 15
 20
 25
 30
 35
 40

atgatagggtt	tgtgtccttt	tcatgatgaa	aagacacctt	catttacagt	ttctgaagat	60
aaacaaatct	gtcattgttt	tggttgtaaa	aaaggtggca	atgtttttca	atttactcaa	120
gaaattaaag	acatatcatt	tgttgaagcg	gttaaagaat	taggtgatag	agttaatggt	180
gctgtagata	ttgaggcaac	acaatctaac	tcaaattgttc	aaattgcttc	tgatgattta	240
caaagtattg	aaatgcatga	gttaatacaa	gaattttatt	attacgcttt	aacaaagaca	300
gtcgaaggcg	aacaagcatt	aacatactta	caagaacgtg	gttttacaga	tgcgcttatt	360
aaagagcgag	gcattggctt	tgaccccgat	agctcacatt	tttgtcatga	ttttcttcaa	420
aaaaagggtt	acgatattga	attagcatat	gaagccggat	tattatcacg	taacgaagaa	480
aatttcagtt	attacgatag	atttcgaaat	cgtattatgt	ttcctttgaa	aaatgcgcaa	540
ggaagaattg	ttggatattc	aggtcgaaca	tataccggtc	aagaaccaa	atacctaagt	600
agtcctgaaa	cgcctatctt	tcaaaaaaga	aagttggtat	ataacttaga	taaagcacgt	660
aatcaatta	gaaaattaga	tgaaattgta	ttactagaag	gttttatgga	tggtataaaa	720
tctgatactg	ctggcttgaa	aaacggttgt	gcaacaatgg	gtacacagtt	gtcagatgaa	780
catattacct	ttatacgaaa	gttaacatca	aatataacat	taatgtttga	tggggatttt	840
gcgggtagtg	aagcaacact	taaaacaggt	caacatttgt	tacagcaagg	gctaaatgta	900
tttggtatatac	aattgccatc	tggtcatggat	ccggatgaat	acattggtaa	gtatggcaac	960
gacgcatttta	ctacttttgt	aaaaaatgac	aaaaagtcac	ttgcacatta	taaagtaagt	1020
atattaaaag	atgaaattgc	acataatgac	ctttcatatg	aacgttattt	gaaagaactg	1080
agtcatgaca	tttcacttat	gaagtcacac	attctgcaac	aaaaggctat	aatgatgtt	1140
gcgccattttt	tcaatgttag	tcctgagcag	ttagctaacg	aaatacaatt	caatcaagca	1200
ccagccaatt	attatccaga	agatgagtat	ggcggttatg	atgagtatgg	cggttatatt	1260
gaacctgagc	caattgggtat	ggcacaattt	gacaatttga	gccgtcgaga	aaaagcggag	1320
cgagcattttt	taaaacattt	aatgagagat	aaagatacat	ttttaaatta	ttatgaaagt	1380
gttgataagg	ataacttcac	aaatcagcat	tttaaatatg	tattcgaagt	cttacatgat	1440
ttttatgcgg	aaaatgatca	atataatatac	agtgatgctg	tgacgtatgt	taattcaaat	1500
gagttgagag	aaacactaat	tagcttagaa	caatataatt	tgaatggcga	accatattgaa	1560
aatgaaattg	atgattatgt	caatgtttat	aatgaaaaag	gacaagaaac	aattgagtc	1620
ttgaatcata	aattaaggga	agctacaagg	attggcgatg	tagaattaca	aaaatactat	1680
ttacagcaaa	ttgttgctaa	gaataaagaa	cgcattgtag			1719

The amino acid sequence of primase encoded by *S. aureus dnaG*
 corresponds to SEQ. ID. No. 16 as follows:

45
 50
 55

Met	Ile	Gly	Leu	Cys	Pro	Phe	His	Asp	Glu	Lys	Thr	Pro	Ser	Phe	Thr
1				5					10					15	
Val	Ser	Glu	Asp	Lys	Gln	Ile	Cys	His	Cys	Phe	Gly	Cys	Lys	Lys	Gly
			20					25					30		
Gly	Asn	Val	Phe	Gln	Phe	Thr	Gln	Glu	Ile	Lys	Asp	Ile	Ser	Phe	Val
		35					40					45			
Glu	Ala	Val	Lys	Glu	Leu	Gly	Asp	Arg	Val	Asn	Val	Ala	Val	Asp	Ile
	50					55						60			

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	Glu	Ala	Thr	Gln	Ser	Asn	Ser	Asn	Val	Gln	Ile	Ala	Ser	Asp	Asp	Leu
	65					70					75					80
5	Gln	Met	Ile	Glu	Met	His	Glu	Leu	Ile	Gln	Glu	Phe	Tyr	Tyr	Tyr	Ala
					85					90					95	
	Leu	Thr	Lys	Thr	Val	Glu	Gly	Glu	Gln	Ala	Leu	Thr	Tyr	Leu	Gln	Glu
				100					105					110		
10	Arg	Gly	Phe	Thr	Asp	Ala	Leu	Ile	Lys	Glu	Arg	Gly	Ile	Gly	Phe	Ala
			115					120					125			
	Pro	Asp	Ser	Ser	His	Phe	Cys	His	Asp	Phe	Leu	Gln	Lys	Lys	Gly	Tyr
15		130					135					140				
	Asp	Ile	Glu	Leu	Ala	Tyr	Glu	Ala	Gly	Leu	Leu	Ser	Arg	Asn	Glu	Glu
	145					150					155					160
20	Asn	Phe	Ser	Tyr	Tyr	Asp	Arg	Phe	Arg	Asn	Arg	Ile	Met	Phe	Pro	Leu
					165					170					175	
	Lys	Asn	Ala	Gln	Gly	Arg	Ile	Val	Gly	Tyr	Ser	Gly	Arg	Thr	Tyr	Thr
				180					185					190		
25	Gly	Gln	Glu	Pro	Lys	Tyr	Leu	Asn	Ser	Pro	Glu	Thr	Pro	Ile	Phe	Gln
			195					200					205			
	Lys	Arg	Lys	Leu	Leu	Tyr	Asn	Leu	Asp	Lys	Ala	Arg	Lys	Ser	Ile	Arg
30		210					215					220				
	Lys	Leu	Asp	Glu	Ile	Val	Leu	Leu	Glu	Gly	Phe	Met	Asp	Val	Ile	Lys
	225					230					235					240
35	Ser	Asp	Thr	Ala	Gly	Leu	Lys	Asn	Val	Val	Ala	Thr	Met	Gly	Thr	Gln
					245					250					255	
	Leu	Ser	Asp	Glu	His	Ile	Thr	Phe	Ile	Arg	Lys	Leu	Thr	Ser	Asn	Ile
				260					265					270		
40	Thr	Leu	Met	Phe	Asp	Gly	Asp	Phe	Ala	Gly	Ser	Glu	Ala	Thr	Leu	Lys
			275					280					285			
	Thr	Gly	Gln	His	Leu	Leu	Gln	Gln	Gly	Leu	Asn	Val	Phe	Val	Ile	Gln
45		290					295					300				
	Leu	Pro	Ser	Gly	Met	Asp	Pro	Asp	Glu	Tyr	Ile	Gly	Lys	Tyr	Gly	Asn
	305					310					315					320
50	Asp	Ala	Phe	Thr	Thr	Phe	Val	Lys	Asn	Asp	Lys	Lys	Ser	Phe	Ala	His
					325					330					335	
	Tyr	Lys	Val	Ser	Ile	Leu	Lys	Asp	Glu	Ile	Ala	His	Asn	Asp	Leu	Ser
				340					345					350		
55	Tyr	Glu	Arg	Tyr	Leu	Lys	Glu	Leu	Ser	His	Asp	Ile	Ser	Leu	Met	Lys
			355					360					365			
	Ser	Ser	Ile	Leu	Gln	Gln	Lys	Ala	Ile	Asn	Asp	Val	Ala	Pro	Phe	Phe
60		370					375					380				
	Asn	Val	Ser	Pro	Glu	Gln	Leu	Ala	Asn	Glu	Ile	Gln	Phe	Asn	Gln	Ala
	385					390					395					400

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	Pro	Ala	Asn	Tyr	Tyr	Pro	Glu	Asp	Glu	Tyr	Gly	Gly	Tyr	Asp	Glu	Tyr
					405					410					415	
5	Gly	Gly	Tyr	Ile	Glu	Pro	Glu	Pro	Ile	Gly	Met	Ala	Gln	Phe	Asp	Asn
				420					425						430	
	Leu	Ser	Arg	Arg	Glu	Lys	Ala	Glu	Arg	Ala	Phe	Leu	Lys	His	Leu	Met
			435					440					445			
10	Arg	Asp	Lys	Asp	Thr	Phe	Leu	Asn	Tyr	Tyr	Glu	Ser	Val	Asp	Lys	Asp
		450					455					460				
	Asn	Phe	Thr	Asn	Gln	His	Phe	Lys	Tyr	Val	Phe	Glu	Val	Leu	His	Asp
15	465					470					475					480
	Phe	Tyr	Ala	Glu	Asn	Asp	Gln	Tyr	Asn	Ile	Ser	Asp	Ala	Val	Gln	Tyr
					485					490					495	
20	Val	Asn	Ser	Asn	Glu	Leu	Arg	Glu	Thr	Leu	Ile	Ser	Leu	Glu	Gln	Tyr
				500					505					510		
	Asn	Leu	Asn	Gly	Glu	Pro	Tyr	Glu	Asn	Glu	Ile	Asp	Asp	Tyr	Val	Asn
		515						520					525			
25	Val	Ile	Asn	Glu	Lys	Gly	Gln	Glu	Thr	Ile	Glu	Ser	Leu	Asn	His	Lys
		530					535					540				
	Leu	Arg	Glu	Ala	Thr	Arg	Ile	Gly	Asp	Val	Glu	Leu	Gln	Lys	Tyr	Tyr
30	545					550					555					560
	Leu	Gln	Gln	Ile	Val	Ala	Lys	Asn	Lys	Glu	Arg	Met				
					565					570						

This invention also relates to the *polC* gene of *Streptococcus pyogenes* encoding the α -large subunit. The partial nucleotide sequence of *polC* (α -large) corresponds to SEQ. ID. No. 17 as follows:

	atgtcagatt	tattcgctaa	attgatggac	cagatagaaa	tgccacttga	catgagacgt	60
40	tcaagtgcct	tttcatctgc	tgatattatc	gaggtaaagg	tacattcggt	gtcacgcttg	120
	tggaatttc	attttgcctt	tgcagcgggt	ttaccgattg	caacttatcg	tgaattgcat	180
	gacgtttga	taagaacttt	tgaggcggct	gacattaagg	taacctttga	catccaagct	240
	gctcaggtgg	attattcaga	tgatctgctt	caagcttatt	accaagaagc	ttttgagcat	300
	gcaccgtgta	atagtgcctag	ttttaaatct	tctttctcaa	agctcaaagt	gacttatgag	360
45	gatgacaaac	tcattattgc	agcgccagggt	tttgtgaata	acgatcattt	tagaaacaat	420
	catctgccta	atctggtcaa	gcaattagaa	gcctttggct	ttggcatctt	gaccatagat	480
	atggtgtcag	atcaggaaat	gactgagcat	ttgaccaaga	attttgtttc	cagtcgtcag	540
	gctcttgtga	aaaaggctgt	gcaggataat	ttggaagccc	aaaaatctct	tgaagccatg	600
	atgccaccag	ttgaggaagc	cacacctgct	cctaagtttg	actacaagga	acgagcagct	660
	aagcgtcagg	cagggtttga	aaaagcaacc	atcacaccaa	tgattgagat	tgagaccgaa	720
50	gaaaaccgga	ttgtctttga	gggtatgggt	tttgacgtgg	agcgtaaaac	gactaggaca	780
	ggtcgccata	tcatacaact	taaaatgaca	gactatacct	cctcgtttgc	tctccaaaaa	840
	tggtgctaaag	acgatgagga	gtcccgtaaa	tttgatatga	ttgctaaggg	agcttggtta	900
	cgggtacaag	ggaatattga	gaccaatcct	tttacgaaga	gtctcaccat	gaatgtccag	960
	cagggtcaaa	aaattgtccg	tcattgagcgc	aaagacctga	tgccagaagg	gcaaaagcgg	1020
55	gtcgaacttc	atgcccacac	caatatgtct	accatggatg	ccttaccgac	agtagaaagc	1080
	ttgattgata	cggcagccaa	gtggggacac	aaggcgattg	ctatcaccga	ccatgctaat	1140
	gtgcaaagtt	ttcctcatgg	ctaccatagg	gctcgcaaag	ctgggattaa	ggctattttt	1200
	ggcctagaag	ccaatattgt	tgaggacaag	gtgcctattt	cttatgaacc	tgttgatatg	1260
	gatttgcaag	aagccacctt	tgtggtcttt	gacgtggaaa	ccacaggtct	atctgctatg	1320
60	aataatgacc	tgattcagat	tgcggcttcc	aaaatgttta	aaggaaatat	tgtagagcag	1380

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	tttgatgaat	tcattgatcc	tgggcaccc	ctttcagcct	ttaccaccga	attgacagga	1440
	attaccgata	agcattttgca	gggcgcacaag	ccatttgggta	ctgtccctaaa	agctttttcag	1500
	gacttttgca	aagatagtat	cttgggttgcc	cacaacgcca	gttttgacgt	gggctttatg	1560
5	aacgcgaatt	atgaacgcca	cgacttgccc	aaaatcacac	agcctgtgat	tgatacctta	1620
	gaatttgcta	gaaacttgta	tccctgagtac	aagcgtcacg	gtttgggacc	gctcaccga	1680
	cgtttccaag	tgagtctaga	ccaccatcat	atggccaatt	acgacgcgga	agccacagga	1740
	cgtcttttgt	ttatttttct	aaaagatgcc	agagaaaagc	atggcatcaa	aaatcttttg	1800
	caactcaata	cagatttggg	ggctgaggat	tcttacaaaa	aagcgcggat	taagcatgcg	1860
10	actatctatg	tgcaaaatca	gggttgggtct	aaaaatatgt	ttaagttggg	cagcctttcc	1920
	aatatcaaat	attttgaagg	gggtgccgcgt	attccaagaa	cgtctctaga	tgctcacaga	1980
	gagggtttgt	tacttggtac	agcttgttct	gacggcgagg	tttttgatgc	cgttctgact	2040
	aaaggaattg	atgcagcggg	tgatttggct	aggtattatg	attttatcga	aatcatgccca	2100
	ccagccattt	accagccatt	gggtgtccgt	gaattaatca	aagatcaagc	aggtattgag	2160
15	cagggtgattc	gtgacctcat	tgaagtaggg	aaacgagcta	agaaacctgt	gcttgccact	2220
	gggaatgtgc	attatctaga	gcctgaagaa	gagatttacc	gtgaaattat	tggtcgtagt	2280
	cttggtcagg	gtgccatgat	taatagaaca	atcgccgtg	gggaaggggc	acagcctgct	2340
	cctctaccta	aagcgcactt	tagaacaacc	aatgaaatgc	tggatgagtt	tgcttttctt	2400
	ggaaaagacc	tcgcttatca	agtagttgtg	caaaatactc	aggattttgc	ggaccgtatt	2460
20	gaggaagtgg	aagtgggtta	gggcgatcct	tacaccccg	atattgataa	ggccgaagag	2520
	acggttgccg	aattaaccta	tcaaaaagcc	tttgaaattt	atggtaatcc	tctccagat	2580
	attattgatt	tacgcattga	aaaagagtta	acctctatct	tggggaacgg	ttttgctgtg	2640
	atttatctcg	cttcccaa	gcttgttaac	cggctcaaatg	agcgaaggct	cctagttggg	2700
	tctaggggat	ctgtagggtc	tagctttgtc	gccaccatga	ttgggattac	tgaggttaat	2760
25	cctatgccgc	ctcactacgt	ttgcccgtcc	tgccaacatt	ctgaatttat	cacagatggg	2820
	tcagttggat	ctggctatga	tttgccctaat	aaaccctgtc	cgaatgtgg	cacccttat	2880
	caaaaagatg	ggcaagacat	tccctttgag	acctttcttg	ggtttgatgg	ggataagggtg	2940
	cccgatattg	atttgaactt	ctctgggtgat	gaccagccca	gtgcccattt	ggatgtccga	3000
	gatatttttg	gtgacgaata	cgctttctgt	gctggaacag	ttggtaccgt	agcagaaaaa	3060
30	acagcttatg	gatttgtcaa	aggctatgaa	cgcgactatg	gcaagttcta	tcgtgatgct	3120
	gaggtggatc	gtctagcagc	agggtgctgt	gggtgtgaa	gaacgactgg	gcagcaccct	3180
	ggggggattg	ttgttattcc	taattacatg	gatgtttatg	attttacccc	cgtgcaatat	3240
	ccagccgatg	atgtaacggc	ttcttggcag	acaactcact	ttaaactcca	tgatattgat	3300
	gaaaacgtct	tataaactga	tatcctaggg	catgatgatc	cgaccatgat	tcgtaaactt	3360
35	caggatttat	cgggcattga	tccctattact	attcctgctg	atgatccggg	agttatggct	3420
	ctcttttctg	ggacagaggt	tttgggcgtt	accccggaa	aaattgggac	accgactggg	3480
	atgctaggca	ttccagaatt	tgggaaccaac	tttgttcgcg	gcatgggtta	tgagacgcat	3540
	ccgaccactt	ttgcggagct	tttgagttg	tctgactat	ctcatggaac	cgatgtttgg	3600
	cttggtaatg	cacaagattt	gatttaaagaa	ggcattgcaa	ccctaaaaaac	cgttatcggg	3660
40	tgctgtgacg	acatcatggg	ttacctcatg	cacgcaggct	tagaaccaaa	aatggccttt	3720
	accattatgg	agcgtgtgcg	taagggatta	tggctaaaaa	tttctgagga	agaacgtaat	3780
	ggctatattg	atgccatgcg	agaaaaacaat	gtgcccga	ggtacattga	atcgtgtgga	3840
	aaaatcaagt	acatgttccc	taaagcccat	cgcgcagctt	atgttttgat	ggcccttcgg	3900
	gtggcttatt	tcaagggtgca	ccaccccat	atgtattatt	gtgcttattt	ctctattcgt	3960
45	gcgaaggcct	ttgaattaaa	aaccatgagt	gggtgtttag	atgctgttaa	agcaagaatg	4020
	gaagatatta	ctataaaacg	taaaaataat	gaagccacca	atgtggaaaa	tgacctcttt	4080
	acaaccttgg	agattgtcaa	cgaaatgtta	gaacgcggct	ttaagtttgg	caaattagac	4140
	ctttacaaaa	gtgatgctat	agaattccaa	atcaaggag	atacccttat	ccctccattt	4200
	atagcgttag	aaggtctggg	tgaaaacgtg	gccaaagcaa	tcgttaaagc	tcgtcaagaa	4260
50	ggcgaattcc	tctctaaaa	ggaattgcgt	aaacgagcg	gggcatcgct	aacgctcggt	4320
	gagaaaatgg	atgagatggg	tatttttagga	aatatgccag	aagataatca	attaagttct	4380
	tttgatgact	ttttc					4395

The encoded α -large subunit has an amino acid sequence corresponding to SEQ. ID.

No. 18 as follows:

55

Met Ser Asp Leu Phe Ala Lys Leu Met Asp Gln Ile Glu Met Pro Leu
1 5 10 15

60

Asp Met Arg Arg Ser Ser Ala Phe Ser Ser Ala Asp Ile Ile Glu Val
20 25 30

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	Lys	Val	His	Ser	Val	Ser	Arg	Leu	Trp	Glu	Phe	His	Phe	Ala	Phe	Ala	
			35					40					45				
5	Ala	Val	Leu	Pro	Ile	Ala	Thr	Tyr	Arg	Glu	Leu	His	Asp	Arg	Leu	Ile	
		50					55					60					
	Arg	Thr	Phe	Glu	Ala	Ala	Asp	Ile	Lys	Val	Thr	Phe	Asp	Ile	Gln	Ala	
	65					70					75					80	
10	Ala	Gln	Val	Asp	Tyr	Ser	Asp	Asp	Leu	Leu	Gln	Ala	Tyr	Tyr	Gln	Glu	
					85					90					95		
	Ala	Phe	Glu	His	Ala	Pro	Cys	Asn	Ser	Ala	Ser	Phe	Lys	Ser	Ser	Phe	
15				100					105					110			
	Ser	Lys	Leu	Lys	Val	Thr	Tyr	Glu	Asp	Asp	Lys	Leu	Ile	Ile	Ala	Ala	
			115					120					125				
20	Pro	Gly	Phe	Val	Asn	Asn	Asp	His	Phe	Arg	Asn	Asn	His	Leu	Pro	Asn	
		130					135					140					
	Leu	Val	Lys	Gln	Leu	Glu	Ala	Phe	Gly	Phe	Gly	Ile	Leu	Thr	Ile	Asp	
	145					150					155					160	
25	Met	Val	Ser	Asp	Gln	Glu	Met	Thr	Glu	His	Leu	Thr	Lys	Asn	Phe	Val	
					165					170					175		
	Ser	Ser	Arg	Gln	Ala	Leu	Val	Lys	Lys	Ala	Val	Gln	Asp	Asn	Leu	Glu	
30				180					185					190			
	Ala	Gln	Lys	Ser	Leu	Glu	Ala	Met	Met	Pro	Pro	Val	Glu	Glu	Ala	Thr	
			195					200					205				
35	Pro	Ala	Pro	Lys	Phe	Asp	Tyr	Lys	Glu	Arg	Ala	Ala	Lys	Arg	Gln	Ala	
		210					215					220					
	Gly	Phe	Glu	Lys	Ala	Thr	Ile	Thr	Pro	Met	Ile	Glu	Ile	Glu	Thr	Glu	
	225					230					235					240	
40	Glu	Asn	Arg	Ile	Val	Phe	Glu	Gly	Met	Val	Phe	Asp	Val	Glu	Arg	Lys	
					245					250					255		
	Thr	Thr	Arg	Thr	Gly	Arg	His	Ile	Ile	Asn	Phe	Lys	Met	Thr	Asp	Tyr	
45				260					265					270			
	Thr	Ser	Ser	Phe	Ala	Leu	Gln	Lys	Trp	Ala	Lys	Asp	Asp	Glu	Glu	Leu	
			275					280					285				
50	Arg	Lys	Phe	Asp	Met	Ile	Ala	Lys	Gly	Ala	Trp	Leu	Arg	Val	Gln	Gly	
		290					295					300					
	Asn	Ile	Glu	Thr	Asn	Pro	Phe	Thr	Lys	Ser	Leu	Thr	Met	Asn	Val	Gln	
	305					310					315					320	
55	Gln	Val	Lys	Glu	Ile	Val	Arg	His	Glu	Arg	Lys	Asp	Leu	Met	Pro	Glu	
					325					330				335			
	Gly	Gln	Lys	Arg	Val	Glu	Leu	His	Ala	His	Thr	Asn	Met	Ser	Thr	Met	
60				340					345					350			
	Asp	Ala	Leu	Pro	Thr	Val	Glu	Ser	Leu	Ile	Asp	Thr	Ala	Ala	Lys	Trp	
			355					360					365				

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	Gly	His	Lys	Ala	Ile	Ala	Ile	Thr	Asp	His	Ala	Asn	Val	Gln	Ser	Phe	
	370						375					380					
5	Pro	His	Gly	Tyr	His	Arg	Ala	Arg	Lys	Ala	Gly	Ile	Lys	Ala	Ile	Phe	
	385					390					395					400	
	Gly	Leu	Glu	Ala	Asn	Ile	Val	Glu	Asp	Lys	Val	Pro	Ile	Ser	Tyr	Glu	
					405					410					415		
10	Pro	Val	Asp	Met	Asp	Leu	His	Glu	Ala	Thr	Tyr	Val	Val	Phe	Asp	Val	
				420					425					430			
	Glu	Thr	Thr	Gly	Leu	Ser	Ala	Met	Asn	Asn	Asp	Leu	Ile	Gln	Ile	Ala	
15				435				440					445				
	Ala	Ser	Lys	Met	Phe	Lys	Gly	Asn	Ile	Val	Glu	Gln	Phe	Asp	Glu	Phe	
		450					455					460					
20	Ile	Asp	Pro	Gly	His	Pro	Leu	Ser	Ala	Phe	Thr	Thr	Glu	Leu	Thr	Gly	
	465					470					475					480	
	Ile	Thr	Asp	Lys	His	Leu	Gln	Gly	Ala	Lys	Pro	Leu	Val	Thr	Val	Leu	
					485					490					495		
25	Lys	Ala	Phe	Gln	Asp	Phe	Cys	Lys	Asp	Ser	Ile	Leu	Val	Ala	His	Asn	
				500					505					510			
	Ala	Ser	Phe	Asp	Val	Gly	Phe	Met	Asn	Ala	Asn	Tyr	Glu	Arg	His	Asp	
30			515					520					525				
	Leu	Pro	Lys	Ile	Thr	Gln	Pro	Val	Ile	Asp	Thr	Leu	Glu	Phe	Ala	Arg	
		530					535					540					
35	Asn	Leu	Tyr	Pro	Glu	Tyr	Lys	Arg	His	Gly	Leu	Gly	Pro	Leu	Thr	Lys	
	545					550					555					560	
	Arg	Phe	Gln	Val	Ser	Leu	Asp	His	His	His	Met	Ala	Asn	Tyr	Asp	Ala	
					565					570					575		
40	Glu	Ala	Thr	Gly	Arg	Leu	Leu	Phe	Ile	Phe	Leu	Lys	Asp	Ala	Arg	Glu	
				580					585					590			
	Lys	His	Gly	Ile	Lys	Asn	Leu	Leu	Gln	Leu	Asn	Thr	Asp	Leu	Val	Ala	
45			595					600					605				
	Glu	Asp	Ser	Tyr	Lys	Lys	Ala	Arg	Ile	Lys	His	Ala	Thr	Ile	Tyr	Val	
		610					615					620					
50	Gln	Asn	Gln	Val	Gly	Leu	Lys	Asn	Met	Phe	Lys	Leu	Val	Ser	Leu	Ser	
	625					630					635					640	
	Asn	Ile	Lys	Tyr	Phe	Glu	Gly	Val	Pro	Arg	Ile	Pro	Arg	Thr	Val	Leu	
					645					650					655		
55	Asp	Ala	His	Arg	Glu	Gly	Leu	Leu	Leu	Gly	Thr	Ala	Cys	Ser	Asp	Gly	
				660					665					670			
	Glu	Val	Phe	Asp	Ala	Val	Leu	Thr	Lys	Gly	Ile	Asp	Ala	Ala	Val	Asp	
60			675					680					685				
	Leu	Ala	Arg	Tyr	Tyr	Asp	Phe	Ile	Glu	Ile	Met	Pro	Pro	Ala	Ile	Tyr	
		690					695					700					

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	Gln	Pro	Leu	Val	Val	Arg	Glu	Leu	Ile	Lys	Asp	Gln	Ala	Gly	Ile	Glu	
	705					710					715					720	
5	Gln	Val	Ile	Arg	Asp	Leu	Ile	Glu	Val	Gly	Lys	Arg	Ala	Lys	Lys	Pro	
					725					730					735		
	Val	Leu	Ala	Thr	Gly	Asn	Val	His	Tyr	Leu	Glu	Pro	Glu	Glu	Glu	Ile	
				740					745					750			
10	Tyr	Arg	Glu	Ile	Ile	Val	Arg	Ser	Leu	Gly	Gln	Gly	Ala	Met	Ile	Asn	
			755					760					765				
	Arg	Thr	Ile	Gly	Arg	Gly	Glu	Gly	Ala	Gln	Pro	Ala	Pro	Leu	Pro	Lys	
15							775					780					
	Ala	His	Phe	Arg	Thr	Thr	Asn	Glu	Met	Leu	Asp	Glu	Phe	Ala	Phe	Leu	
	785					790					795					800	
20	Gly	Lys	Asp	Leu	Ala	Tyr	Gln	Val	Val	Val	Gln	Asn	Thr	Gln	Asp	Phe	
					805					810					815		
	Ala	Asp	Arg	Ile	Glu	Glu	Val	Glu	Val	Val	Lys	Gly	Asp	Leu	Tyr	Thr	
				820				825						830			
25	Pro	Tyr	Ile	Asp	Lys	Ala	Glu	Glu	Thr	Val	Ala	Glu	Leu	Thr	Tyr	Gln	
			835					840					845				
	Lys	Ala	Phe	Glu	Ile	Tyr	Gly	Asn	Pro	Leu	Pro	Asp	Ile	Ile	Asp	Leu	
30			850				855					860					
	Arg	Ile	Glu	Lys	Glu	Leu	Thr	Ser	Ile	Leu	Gly	Asn	Gly	Phe	Ala	Val	
	865					870					875					880	
35	Ile	Tyr	Leu	Ala	Ser	Gln	Met	Leu	Val	Asn	Arg	Ser	Asn	Glu	Arg	Gly	
					885					890					895		
	Tyr	Leu	Val	Gly	Ser	Arg	Gly	Ser	Val	Gly	Ser	Ser	Phe	Val	Ala	Thr	
				900					905					910			
40	Met	Ile	Gly	Ile	Thr	Glu	Val	Asn	Pro	Met	Pro	Pro	His	Tyr	Val	Cys	
			915					920					925				
	Pro	Ser	Cys	Gln	His	Ser	Glu	Phe	Ile	Thr	Asp	Gly	Ser	Val	Gly	Ser	
45			930				935					940					
	Gly	Tyr	Asp	Leu	Pro	Asn	Lys	Pro	Cys	Pro	Lys	Cys	Gly	Thr	Pro	Tyr	
	945					950					955					960	
50	Gln	Lys	Asp	Gly	Gln	Asp	Ile	Pro	Phe	Glu	Thr	Phe	Leu	Gly	Phe	Asp	
					965					970					975		
	Gly	Asp	Lys	Val	Pro	Asp	Ile	Asp	Leu	Asn	Phe	Ser	Gly	Asp	Asp	Gln	
				980					985					990			
55	Pro	Ser	Ala	His	Leu	Asp	Val	Arg	Asp	Ile	Phe	Gly	Asp	Glu	Tyr	Ala	
			995				1000						1005				
	Phe	Arg	Ala	Gly	Thr	Val	Gly	Thr	Val	Ala	Glu	Lys	Thr	Ala	Tyr	Gly	
60			1010				1015					1020					
	Phe	Val	Lys	Gly	Tyr	Glu	Arg	Asp	Tyr	Gly	Lys	Phe	Tyr	Arg	Asp	Ala	
	1025					1030					1035					1040	

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	Glu Val Asp Arg Leu Ala Ala Gly Ala Ala Gly Val Lys Arg Thr Thr	
	1045	1050 1055
5	Gly Gln His Pro Gly Gly Ile Val Val Ile Pro Asn Tyr Met Asp Val	
	1060	1065 1070
	Tyr Asp Phe Thr Pro Val Gln Tyr Pro Ala Asp Asp Val Thr Ala Ser	
	1075	1080 1085
10	Trp Gln Thr Thr His Phe Asn Phe His Asp Ile Asp Glu Asn Val Leu	
	1090	1095 1100
	Lys Leu Asp Ile Leu Gly His Asp Asp Pro Thr Met Ile Arg Lys Leu	
	1105	1110 1115 1120
15	Gln Asp Leu Ser Gly Ile Asp Pro Ile Thr Ile Pro Ala Asp Asp Pro	
	1125	1130 1135
20	Gly Val Met Ala Leu Phe Ser Gly Thr Glu Val Leu Gly Val Thr Pro	
	1140	1145 1150
	Glu Gln Ile Gly Thr Pro Thr Gly Met Leu Gly Ile Pro Glu Phe Gly	
	1155	1160 1165
25	Thr Asn Phe Val Arg Gly Met Val Asn Glu Thr His Pro Thr Thr Phe	
	1170	1175 1180
	Ala Glu Leu Leu Gln Leu Ser Gly Leu Ser His Gly Thr Asp Val Trp	
	1185	1190 1195 1200
30	Leu Gly Asn Ala Gln Asp Leu Ile Lys Glu Gly Ile Ala Thr Leu Lys	
	1205	1210 1215
35	Thr Val Ile Gly Cys Arg Asp Asp Ile Met Val Tyr Leu Met His Ala	
	1220	1225 1230
	Gly Leu Glu Pro Lys Met Ala Phe Thr Ile Met Glu Arg Val Arg Lys	
	1235	1240 1245
40	Gly Leu Trp Leu Lys Ile Ser Glu Glu Glu Arg Asn Gly Tyr Ile Asp	
	1250	1255 1260
	Ala Met Arg Glu Asn Asn Val Pro Asp Trp Tyr Ile Glu Ser Cys Gly	
	1265	1270 1275 1280
45	Lys Ile Lys Tyr Met Phe Pro Lys Ala His Ala Ala Ala Tyr Val Leu	
	1285	1290 1295
50	Met Ala Leu Arg Val Ala Tyr Phe Lys Val His His Pro Ile Met Tyr	
	1300	1305 1310
	Tyr Cys Ala Tyr Phe Ser Ile Arg Ala Lys Ala Phe Glu Leu Lys Thr	
	1315	1320 1325
55	Met Ser Gly Gly Leu Asp Ala Val Lys Ala Arg Met Glu Asp Ile Thr	
	1330	1335 1340
	Ile Lys Arg Lys Asn Asn Glu Ala Thr Asn Val Glu Asn Asp Leu Phe	
	1345	1350 1355 1360
60	Thr Thr Leu Glu Ile Val Asn Glu Met Leu Glu Arg Gly Phe Lys Phe	
	1365	1370 1375

Gly Lys Leu Asp Leu Tyr Lys Ser Asp Ala Ile Glu Phe Gln Ile Lys
 1380 1385 1390
 5 Gly Asp Thr Leu Ile Pro Pro Phe Ile Ala Leu Glu Gly Leu Gly Glu
 1395 1400 1405
 Asn Val Ala Lys Gln Ile Val Lys Ala Arg Gln Glu Gly Glu Phe Leu
 1410 1415 1420
 10 Ser Lys Met Glu Leu Arg Lys Arg Gly Gly Ala Ser Ser Thr Leu Val
 1425 1430 1435 1440
 Glu Lys Met Asp Glu Met Gly Ile Leu Gly Asn Met Pro Glu Asp Asn
 1445 1450 1455
 15 Gln Leu Ser Leu Phe Asp Asp Phe Phe
 1460 1465

20 The present invention also relates to the *dnaE* gene of *Streptococcus pyogenes* encoding the α -small subunit. The partial nucleotide sequence of the *dnaE* gene corresponds to SEQ. ID. No. 19 as follows:

25 atgtttgctc aacttgatac taaaactgta tactcattta tggatagttt aattgactta 60
 aatcattatt ttgaacgagc aaagcaattt gggtaccaca ccataggaat catggataag 120
 gataatcttt atggtgctta ccattttatt aaaggttgtc aaaaaaatgg actgcagcca 180
 gtttttaggtt tggaaataga gattctctat caagagcggc aggtgctcct taacttaatc 240
 gccagaata cacaaggcta tcatcagctt ttaaaaattt ccacggcaaa aatgtctggc 300
 aagcttcata tggattactt ctgccaacat ttggaaggga tagcgggttat tattcctagt 360
 aagggttggg gcgatacatt agtggtcctt ttgtactact atatgggtgt tgatcagtat 420
 30 actgatttat ctcatatgga ttctaaggag cagcttatac ccctaaggac agttcgttat 480
 tttgcgcaag atgatatgga aacctgcac atgttgcatt ccattcgaga taacctcagt 540
 ctggcagaga cccctgtggt agaaagtgat caagagttag cagattgtca acaactaacc 600
 gccttctatc aaacacactg ccctcaagct ctacagaatt tagaagactt agtgtcagga 660
 atctattatg atttcgatac aaatttaaaa ttgcctcatt ttaatagaga taagtctgcc 720
 35 aagcaagaat tgcaagactt gactgaggct gggttgaagg aaaaaggatt gtggaaagag 780
 ccttatcaat cgcgcttact acatgaattg gtcattattt ctgacatggg ctttgatgat 840
 tattttttga ttgtgtggga tttacttcgc tttggacgca gtaaaggcta ttatatggga 900
 atgggacgtg gctcggcggc aggtagtcta gtggttatg ctctgaacat tacagggatt 960
 gatccagttc aacatgattt gctatttgag cgctttttta acaaagaacg ttatagcatg 1020
 40 cctgatattg atatcgatct tccagatatt taccgttcag aatttctacg gtatgtccga 1080
 aatcgttatg gttagcgacca ttccggcgcaa attgtgacct tttcaacctt tggccaggct 1140
 attcgtgatg ttttcaaacg gttcgggggtt ccagaatacg aactgactaa tctcactaaa 1200
 aaaattgggt ttaaagatag cttggctact gtctatgaaa agtcaatctc ttttaggcag 1260
 gttattaata gtagaactga atttcaaaag gcttttgcca ttgccaagcg tategaagga 1320
 45 aatccaagac aaacgtccat tcacgcagct ggtattgtga tgagtgatga tgccttgacc 1380
 aatcatattc ctctaaaatc gggcgatgac atgatgatca cccagtatga tgctcatgcy 1440
 gtcgaagcta atggcctgtt aaaaatggat tttttgggtt taagaaattt gacctttgtt 1500
 caaaaaatgc aagagaaggt tgctaaagac tacgggtgtc agattgatat tacagccatt 1560
 gatttagaag acccgcaaac gttggcactt tttgctaaag gggataccaa gggaattttc 1620
 50 caatttgaac aaaatggtgc tattaatctt ttaaaacgga ttaagccaca acgttttgaa 1680
 gaaattgttg ccactaccag tctaaataga ccaggggcaa gtgactatac cactaatttc 1740
 attaaagaaa gagaaggaca agaaaaaatt gatttgattg atcctgtgat tgctccattt 1800
 ttagagccaa cttacggtat tatgctttat caagaacaag ttatgcagat tgcacagggt 1860
 tatgctggtt ttacgttagg caaggccgac ttgttaaggc gtgccatgtc taaaaaaaat 1920
 55 ctacaagaaa tgcaaaaaat ggaagaagac tttattgctt ctgctaagca cctagggaga 1980
 gctgaagaaa cagctagagg actttttaaa cggatggaaa aatttgcagg ttatggtttt 2040
 aaccgcagcc atgcctttgc ctattcagct ttatgctttt aattggctta tttcaaagcc 2100
 cattaccggg ctgtttttta cgatatcatg atgaattatt ctacagtgat ctatatcaca 2160
 gatgctctag aatcagattt tcaagtagcg caagttaacca ttaatagtat tccttacact 2220
 60 gataaaattg aagctagcaa gatttacatg gggctgaaaa atattaaggg gttgccaagg 2280

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5 gattttgctt attggattat cgagcaaaga ccatttaata gcgtagagga ttttctcact 2340
 agaactccag aaaaatatca aaaaaaggtt ttccttgagc ctctgataaa aataggtctg 2400
 tttgattgct ttgagcctaa ccgtaaaaaa attctggaca atttggatgg tttactggta 2460
 tttgttaatg agcttggttc tcttttttca gattcttctt ttagttgggt agatacgaaa 2520
 gattactcag taactgaaaa atattctttg gaacaggaga tcgttggagt tggcatgagc 2580
 aagcatcctt taattgatat tgctgagaaa agtaccctaa cttttactcc tatttcacag 2640
 ttagtcaaag aaagcgaagc agtcgtactg attcaaatag atagcattag gatcattaga 2700
 accaaaacaa gtgggcagca aatggctttt ttaagtgtga atgacactaa gaaaaagctc 2760
 10 gatgtcacac tttttccaca agagtatgcc atttataaag accaattaaa agaaggagaa 2820
 ttctattact taaaaggtag aataaaaagaa agagaccatc gactgcagat ggtgtgtcag 2880
 caagtgcaaa tggctattag tcaaaaatat tgggtattag ttgaaaacca tcagtttgat 2940
 tcccaaattt ctgagatttt aggtgccttt ccaggaacga ctccagttgt tattcactat 3000
 caaaaaata aggaaacaat tgcattaact aagattcagg ttcattgtaac agagaattta 3060
 15 aaggaaaaaac ttcgtccttt tgttctgaaa acggtttttc ga 3102

The encoded α -small subunit has an amino acid sequence corresponding to SEQ. ID.
No. 20 as follows:

20 Met Phe Ala Gln Leu Asp Thr Lys Thr Val Tyr Ser Phe Met Asp Ser
 1 5 10 15
 Leu Ile Asp Leu Asn His Tyr Phe Glu Arg Ala Lys Gln Phe Gly Tyr
 20 25 30
 25 His Thr Ile Gly Ile Met Asp Lys Asp Asn Leu Tyr Gly Ala Tyr His
 35 40 45
 Phe Ile Lys Gly Cys Gln Lys Asn Gly Leu Gln Pro Val Leu Gly Leu
 50 55 60
 30 Glu Ile Glu Ile Leu Tyr Gln Glu Arg Gln Val Leu Leu Asn Leu Ile
 65 70 75 80
 Ala Gln Asn Thr Gln Gly Tyr His Gln Leu Leu Lys Ile Ser Thr Ala
 85 90 95
 35 Lys Met Ser Gly Lys Leu His Met Asp Tyr Phe Cys Gln His Leu Glu
 100 105 110
 40 Gly Ile Ala Val Ile Ile Pro Ser Lys Gly Trp Ser Asp Thr Leu Val
 115 120 125
 Val Pro Phe Asp Tyr Tyr Met Gly Val Asp Gln Tyr Thr Asp Leu Ser
 130 135 140
 45 His Met Asp Ser Lys Arg Gln Leu Ile Pro Leu Arg Thr Val Arg Tyr
 145 150 155 160
 Phe Ala Gln Asp Asp Met Glu Thr Leu His Met Leu His Ala Ile Arg
 165 170 175
 50 Asp Asn Leu Ser Leu Ala Glu Thr Pro Val Val Glu Ser Asp Gln Glu
 180 185 190
 55 Leu Ala Asp Cys Gln Gln Leu Thr Ala Phe Tyr Gln Thr His Cys Pro
 195 200 205
 Gln Ala Leu Gln Asn Leu Glu Asp Leu Val Ser Gly Ile Tyr Tyr Asp
 210 215 220
 60

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	Phe	Asp	Thr	Asn	Leu	Lys	Leu	Pro	His	Phe	Asn	Arg	Asp	Lys	Ser	Ala
	225					230					235					240
5	Lys	Gln	Glu	Leu	Gln	Asp	Leu	Thr	Glu	Ala	Gly	Leu	Lys	Glu	Lys	Gly
					245					250					255	
	Leu	Trp	Lys	Glu	Pro	Tyr	Gln	Ser	Arg	Leu	Leu	His	Glu	Leu	Val	Ile
				260					265					270		
10	Ile	Ser	Asp	Met	Gly	Phe	Asp	Asp	Tyr	Phe	Leu	Ile	Val	Trp	Asp	Leu
			275					280					285			
	Leu	Arg	Phe	Gly	Arg	Ser	Lys	Gly	Tyr	Tyr	Met	Gly	Met	Gly	Arg	Gly
15		290					295					300				
	Ser	Ala	Ala	Gly	Ser	Leu	Val	Ala	Tyr	Ala	Leu	Asn	Ile	Thr	Gly	Ile
	305					310					315					320
20	Asp	Pro	Val	Gln	His	Asp	Leu	Leu	Phe	Glu	Arg	Phe	Leu	Asn	Lys	Glu
					325					330					335	
	Arg	Tyr	Ser	Met	Pro	Asp	Ile	Asp	Ile	Asp	Leu	Pro	Asp	Ile	Tyr	Arg
				340					345					350		
25	Ser	Glu	Phe	Leu	Arg	Tyr	Val	Arg	Asn	Arg	Tyr	Gly	Ser	Asp	His	Ser
			355					360					365			
	Ala	Gln	Ile	Val	Thr	Phe	Ser	Thr	Phe	Gly	Pro	Lys	Gln	Ala	Ile	Arg
30							375					380				
	Asp	Val	Phe	Lys	Arg	Phe	Gly	Val	Pro	Glu	Tyr	Glu	Leu	Thr	Asn	Leu
	385					390					395					400
35	Thr	Lys	Lys	Ile	Gly	Phe	Lys	Asp	Ser	Leu	Ala	Thr	Val	Tyr	Glu	Lys
					405					410					415	
	Ser	Ile	Ser	Phe	Arg	Gln	Val	Ile	Asn	Ser	Arg	Thr	Glu	Phe	Gln	Lys
				420					425					430		
40	Ala	Phe	Ala	Ile	Ala	Lys	Arg	Ile	Glu	Gly	Asn	Pro	Arg	Gln	Thr	Ser
			435					440					445			
	Ile	His	Ala	Ala	Gly	Ile	Val	Met	Ser	Asp	Asp	Ala	Leu	Thr	Asn	His
45							455					460				
	Ile	Pro	Leu	Lys	Ser	Gly	Asp	Asp	Met	Met	Ile	Thr	Gln	Tyr	Asp	Ala
	465					470					475					480
50	His	Ala	Val	Glu	Ala	Asn	Gly	Leu	Leu	Lys	Met	Asp	Phe	Leu	Gly	Leu
					485					490					495	
	Arg	Asn	Leu	Thr	Phe	Val	Gln	Lys	Met	Gln	Glu	Lys	Val	Ala	Lys	Asp
				500					505					510		
55	Tyr	Gly	Cys	Gln	Ile	Asp	Ile	Thr	Ala	Ile	Asp	Leu	Glu	Asp	Pro	Gln
			515					520					525			
	Thr	Leu	Ala	Leu	Phe	Ala	Lys	Gly	Asp	Thr	Lys	Gly	Ile	Phe	Gln	Phe
60							535					540				
	Glu	Gln	Asn	Gly	Ala	Ile	Asn	Leu	Leu	Lys	Arg	Ile	Lys	Pro	Gln	Arg
	545					550					555					560

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	Phe	Glu	Glu	Ile	Val	Ala	Thr	Thr	Ser	Leu	Asn	Arg	Pro	Gly	Ala	Ser	
					565					570					575		
5	Asp	Tyr	Thr	Thr	Asn	Phe	Ile	Lys	Arg	Arg	Glu	Gly	Gln	Glu	Lys	Ile	
					580				585					590			
	Asp	Leu	Ile	Asp	Pro	Val	Ile	Ala	Pro	Ile	Leu	Glu	Pro	Thr	Tyr	Gly	
					595			600					605				
10	Ile	Met	Leu	Tyr	Gln	Glu	Gln	Val	Met	Gln	Ile	Ala	Gln	Val	Tyr	Ala	
		610					615					620					
	Gly	Phe	Thr	Leu	Gly	Lys	Ala	Asp	Leu	Leu	Arg	Arg	Ala	Met	Ser	Lys	
15		625				630					635					640	
	Lys	Asn	Leu	Gln	Glu	Met	Gln	Lys	Met	Glu	Glu	Asp	Phe	Ile	Ala	Ser	
					645					650					655		
20	Ala	Lys	His	Leu	Gly	Arg	Ala	Glu	Glu	Thr	Ala	Arg	Gly	Leu	Phe	Lys	
					660				665					670			
	Arg	Met	Glu	Lys	Phe	Ala	Gly	Tyr	Gly	Phe	Asn	Arg	Ser	His	Ala	Phe	
			675					680					685				
25	Ala	Tyr	Ser	Ala	Leu	Ala	Phe	Gln	Leu	Ala	Tyr	Phe	Lys	Ala	His	Tyr	
		690					695					700					
	Pro	Ala	Val	Phe	Tyr	Asp	Ile	Met	Met	Asn	Tyr	Ser	Ser	Ser	Asp	Tyr	
30		705				710					715					720	
	Ile	Thr	Asp	Ala	Leu	Glu	Ser	Asp	Phe	Gln	Val	Ala	Gln	Val	Thr	Ile	
					725					730					735		
35	Asn	Ser	Ile	Pro	Tyr	Thr	Asp	Lys	Ile	Glu	Ala	Ser	Lys	Ile	Tyr	Met	
				740					745					750			
	Gly	Leu	Lys	Asn	Ile	Lys	Gly	Leu	Pro	Arg	Asp	Phe	Ala	Tyr	Trp	Ile	
			755					760					765				
40	Ile	Glu	Gln	Arg	Pro	Phe	Asn	Ser	Val	Glu	Asp	Phe	Leu	Thr	Arg	Thr	
		770					775					780					
	Pro	Glu	Lys	Tyr	Gln	Lys	Lys	Val	Phe	Leu	Glu	Pro	Leu	Ile	Lys	Ile	
45		785				790					795					800	
	Gly	Leu	Phe	Asp	Cys	Phe	Glu	Pro	Asn	Arg	Lys	Lys	Ile	Leu	Asp	Asn	
					805					810					815		
50	Leu	Asp	Gly	Leu	Leu	Val	Phe	Val	Asn	Glu	Leu	Gly	Ser	Leu	Phe	Ser	
				820					825					830			
	Asp	Ser	Ser	Phe	Ser	Trp	Val	Asp	Thr	Lys	Asp	Tyr	Ser	Val	Thr	Glu	
				835				840					845				
55	Lys	Tyr	Ser	Leu	Glu	Gln	Glu	Ile	Val	Gly	Val	Gly	Met	Ser	Lys	His	
		850					855					860					
	Pro	Leu	Ile	Asp	Ile	Ala	Glu	Lys	Ser	Thr	Gln	Thr	Phe	Thr	Pro	Ile	
60		865				870					875					880	
	Ser	Gln	Leu	Val	Lys	Glu	Ser	Glu	Ala	Val	Val	Leu	Ile	Gln	Ile	Asp	
					885					890						895	

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Ser Ile Arg Ile Ile Arg Thr Lys Thr Ser Gly Gln Gln Met Ala Phe
 900 905 910
 5 Leu Ser Val Asn Asp Thr Lys Lys Lys Leu Asp Val Thr Leu Phe Pro
 915 920 925
 Gln Glu Tyr Ala Ile Tyr Lys Asp Gln Leu Lys Glu Gly Glu Phe Tyr
 930 935 940
 10 Tyr Leu Lys Gly Arg Ile Lys Glu Arg Asp His Arg Leu Gln Met Val
 945 950 955 960
 Cys Gln Gln Val Gln Met Ala Ile Ser Gln Lys Tyr Trp Leu Leu Val
 965 970 975
 15 Glu Asn His Gln Phe Asp Ser Gln Ile Ser Glu Ile Leu Gly Ala Phe
 980 985 990
 Pro Gly Thr Thr Pro Val Val Ile His Tyr Gln Lys Asn Lys Glu Thr
 995 1000 1005
 20 Ile Ala Leu Thr Lys Ile Gln Val Thr Glu Asn Leu Lys Glu Lys Leu
 1010 1015 1020
 25 Arg Pro Phe Val Leu Lys Thr Val Phe Arg
 1025 1030

The present invention also relates to the *hola* gene of *Streptococcus*
pyogenes encoding the δ subunit. The *hola* gene has a nucleotide sequence which
 30 corresponds to SEQ. ID. No. 21 as follows:

atgattgcga tagaaaagat tgaaaaactg agtaaagaaa atttgggtct tataaccctt 60
 gtcacaggag atgacattgg tcagtatagc cagttgaaat cccgcttaat ggagcagatt 120
 35 gcttttgata aggatgattt ggcctattct tactttgata tgtctgaggc cgcttatcag 180
 gatgcagaaa tggatctagt gaggctaccc ttctttgctg agcagaaggt ggttattttt 240
 gaccatttgt tagatatcac gaccaataaa aaaagtttct taaaagaaaa agacctaaag 300
 gcctttgaag cctattttaga aaatccctta gagactactc gactaattat ctttgctcca 360
 ggtaaatggg atagtaagag acggcttggt aagcttttga aacgtgatgc ccttgtttta 420
 40 gaagccaacc ctctgaaaga agcagagcta agaacttatt ttcaaaaata cagtcacaa 480
 ctgggttttag gtttcgagag tgggtgccttt gaccaattac ttttgaaatc aaacgatgat 540
 tttagtcaaa tcatgaaaaa catggccttt ttaaaagcct ataaaaaac gggaaatatt 600
 agcctaactg atattgagca agccattcct aaaagtttac aagataatat ttctgatctg 660
 actagacttg tcctaggagg taaaattgat gcggctagag atttgattca tgatttacgg 720
 45 ttatctggag aagatgacat taaattaatc gctatcatgc taggccaatt tcgcttattt 780
 ttgcagctga ctattcttgc tagagatgta aaaaacgagc aacaactagt gattagttaa 840
 tcagatattc ttgggcggcg ggtaaatcct taccagggtca agtatgcgtt aaaggattct 900
 aggaccttat ctcttgccct tctaacagga gcggtgaaaa ccttgattga gacagattac 960
 cagataaaaa caggacttta tgagaagagt tatctagttg atattgctct cttaaaaatc 1020
 atgactcact ctcaaaaa 1038
 50

The encoded δ subunit has an amino acid sequence corresponding to SEQ. ID. No. 22
 as follows:

55 Met Ile Ala Ile Glu Lys Ile Glu Lys Leu Ser Lys Glu Asn Leu Gly
 1 5 10 15

The present invention also relates to the *holB* gene of *Streptococcus pyogenes* encoding the δ' subunit. The *holB* gene has a nucleotide sequence which corresponds to SEQ. ID. No. 23 as follows:

```

5   atggatttag cgcaaaaagc tctaacggt tatcaagctt ttcagacaat tttaaagaaa 60
   gaccgtctga atcatgctta tcttttttcg ggtgattttg ctaatgaaga aatggctctt 120
   tttttagcta aggtcatctt ttgtgaacag aaaaaggatc agacgccttg cgggcattgt 180
   cgctcttgtc aattgattga acaaggagat ttgtccgatg tgacggtatt ggaaccaaca 240
   gggcaagtga ttaaaacgga tgtggtcaaa gaaatgatgg ctaacttttc tcagacagga 300
10  tatgaaaaca aacgacaagt ttttattatc aaagattgtg acaaaatgca tatcaatgcc 360
   gctaatagct tgctaaaata cattgaggag cctcagggag aagcttacat atttttattg 420
   accaatgatg ataacaaagt gcttccgacc attaaaagtc ggacacaggt ttttcagttt 480
   cctaaaaacg aagcctatct ttaccaattg gcacaagaaa agggattatt aaaccatcag 540
   gctaagctag tagccaaact tgccacaaac accagtcac tagaacgtct gttgcaaacg 600
15  agcaagcttt tagaactgat aactcaagca gagcgttttg tatctatttg gctgaaagat 660
   cagttgcagg catatttagc gttgaaccgt ctggtacagt tagcaactga aaaagaagaa 720
   caagatttag ttttgacctt tttgaccttg ctcttggtgca gagagcgtgc gcaaacgcct 780
   ttgacacaat tggaggctgt ctatcaggct aggcctcatgt ggcagagcaa tgtaatttt 840
   caaaacacat tagaatatat ggtgatgtca gaa 873
20

```

The encoded δ' subunit has an amino acid sequence corresponding to SEQ. ID. No. 24 as follows:

```

Met Asp Leu Ala Gln Lys Ala Pro Asn Val Tyr Gln Ala Phe Gln Thr
  1             5             10             15
25  Ile Leu Lys Lys Asp Arg Leu Asn His Ala Tyr Leu Phe Ser Gly Asp
      20             25             30
30  Phe Ala Asn Glu Glu Met Ala Leu Phe Leu Ala Lys Val Ile Phe Cys
      35             40             45
   Glu Gln Lys Lys Asp Gln Thr Pro Cys Gly His Cys Arg Ser Cys Gln
      50             55             60
35  Leu Ile Glu Gln Gly Asp Phe Ala Asp Val Thr Val Leu Glu Pro Thr
      65             70             75             80
   Gly Gln Val Ile Lys Thr Asp Val Val Lys Glu Met Met Ala Asn Phe
      85             90             95
40  Ser Gln Thr Gly Tyr Glu Asn Lys Arg Gln Val Phe Ile Ile Lys Asp
      100            105            110
   Cys Asp Lys Met His Ile Asn Ala Ala Asn Ser Leu Leu Lys Tyr Ile
      115            120            125
   Glu Glu Pro Gln Gly Glu Ala Tyr Ile Phe Leu Leu Thr Asn Asp Asp
      130            135            140
50  Asn Lys Val Leu Pro Thr Ile Lys Ser Arg Thr Gln Val Phe Gln Phe
      145            150            155            160
   Pro Lys Asn Glu Ala Tyr Leu Tyr Gln Leu Ala Gln Glu Lys Gly Leu
      165            170            175
55  Leu Asn His Gln Ala Lys Leu Val Ala Lys Leu Ala Thr Asn Thr Ser
      180            185            190

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	His	Leu	Glu	Arg	Leu	Leu	Gln	Thr	Ser	Lys	Leu	Leu	Glu	Leu	Ile	Thr
		195						200					205			
5	Gln	Ala	Glu	Arg	Phe	Val	Ser	Ile	Trp	Leu	Lys	Asp	Gln	Leu	Gln	Ala
	210						215					220				
	Tyr	Leu	Ala	Leu	Asn	Arg	Leu	Val	Gln	Leu	Ala	Thr	Glu	Lys	Glu	Glu
	225				230						235				240	
10	Gln	Asp	Leu	Val	Leu	Thr	Leu	Leu	Thr	Leu	Leu	Leu	Ala	Arg	Glu	Arg
				245					250					255		
	Ala	Gln	Thr	Pro	Leu	Thr	Gln	Leu	Glu	Ala	Val	Tyr	Gln	Ala	Arg	Leu
15				260					265					270		
	Met	Trp	Gln	Ser	Asn	Val	Asn	Phe	Gln	Asn	Thr	Leu	Glu	Tyr	Met	Val
		275					280						285			
20	Met	Ser	Glu													
		290														

The present invention also relates to the *dnaX* gene of *Streptococcus pyogenes* encoding the τ subunit. The *dnaX* gene has a nucleotide sequence which corresponds to SEQ. ID. No. 25 as follows:

	atgtatcaag	ctcttttatcg	gaaataccgg	agccaaacgt	ttgacgaaat	ggtggggacaa	60
	tccggttattt	ccacaacttt	aaagcaggca	gttgaatctg	gcaagattag	ccatgcttat	120
30	cttttttcag	gtcctagagg	gactgggaaa	acaagtgcgg	caaagatttt	tgcaaaggcc	180
	atgaattgtc	ctaaccaagt	cgatggtgaa	ccctgtaatc	aatgcgatat	ttgccgagat	240
	atcacgaatg	gaagcttgga	agatgtgatt	gaaattgatg	ctgcctcgaa	taattggtgtt	300
	gatgaaattc	gtgacattcg	agacaaatca	acctatgcgc	caagtcgtgc	gacttacaag	360
	gtttatatta	ttgatgaggt	tcacatgtta	tcaacagggg	cttttaaatgc	gcttttgaaa	420
	actttggaag	aaccgacaga	atgtttgtct	tatcttggca	acaacggaat	gcataaaatt	480
35	ccagccacta	ttttatctcg	tgtgcaacgc	tttgaattca	aagctattaa	gcaaaaagct	540
	attcgagagc	atttagcctg	ggttttggac	aaagaaggta	ttgcctatga	ggtggtatgct	600
	ttaaatctca	ttgcaaggcg	agcagaagga	ggcatgcgtg	atgctttatc	tatttttagat	660
	caggctttga	gcttgtcacc	agataatcag	gtcgccattg	caattgccga	agaaattaca	720
40	ggttctattt	ccataacttg	tctgggtgac	tatgttcgat	atgtctccca	agaacaggct	780
	acgcaagctc	tggcagcctt	agagaccatt	tatgatagtg	ggaagagcat	gagccgcttt	840
	gcgacagatt	tattgacctt	tctgcgtgat	ttattgggtg	ttaaagctgg	cggcgacaat	900
	caacgtcagt	cagctgtttt	tgataccaat	ttgtctctct	cgatagatcg	tatattccaa	960
	atgataacag	ttgttactag	tcatctccct	gaaatcaaaa	agggaaacca	tcctcggatt	1020
	tatgccgaaa	tgatgactat	ccaattagct	cagaaagagc	agattttgtc	ccaagtaaac	1080
45	ttgtcaggag	agttaatctc	agagattgaa	acgctcaaaa	atgagttggc	acaacttaaa	1140
	caacaattgt	cgcagctcca	atcgcgtcct	gattcactgg	caagatctga	taaaacgaaa	1200
	cctaaaacca	caagctacag	ggttgatcgg	gttaccattt	tgaaaatcat	ggaagaaacg	1260
	gttcgaaata	gccaacaatc	tcgacaatat	ctagatgctc	taaaaaatgc	ttggaatgaa	1320
	attctagata	acattttctg	ccaagacaga	gccttattga	tgggctctga	gctgtctcta	1380
50	gcaaatagtg	agaatgcgat	tttggctttc	gaggctgcct	ttaatgcaga	acaagtcatg	1440
	agccgaaata	atcttaatga	tatgtttggt	aacattatga	gtaaagctgc	tggtttttct	1500
	cccaatattc	tggcagtagc	aaggacagat	tttcagcata	ttcgtaagga	atttgctcag	1560
	caaatgaaat	cgcaaaaaga	cagtgttcaa	gaagaacaag	aagtagcgct	tgatattcca	1620
55	gaagggtttg	attttttgct	cgataaaaata	aatactattg	acgac		1665

The encoded τ subunit has an amino acid sequence corresponding to SEQ. ID. No. 26 as follows:

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	Met	Tyr	Gln	Ala	Leu	Tyr	Arg	Lys	Tyr	Arg	Ser	Gln	Thr	Phe	Asp	Glu	
	1				5					10					15		
5	Met	Val	Gly	Gln	Ser	Val	Ile	Ser	Thr	Thr	Leu	Lys	Gln	Ala	Val	Glu	
				20					25					30			
	Ser	Gly	Lys	Ile	Ser	His	Ala	Tyr	Leu	Phe	Ser	Gly	Pro	Arg	Gly	Thr	
			35					40					45				
10	Gly	Lys	Thr	Ser	Ala	Ala	Lys	Ile	Phe	Ala	Lys	Ala	Met	Asn	Cys	Pro	
		50					55					60					
	Asn	Gln	Val	Asp	Gly	Glu	Pro	Cys	Asn	Gln	Cys	Asp	Ile	Cys	Arg	Asp	
15	65					70					75					80	
	Ile	Thr	Asn	Gly	Ser	Leu	Glu	Asp	Val	Ile	Glu	Ile	Asp	Ala	Ala	Ser	
					85					90					95		
20	Asn	Asn	Gly	Val	Asp	Glu	Ile	Arg	Asp	Ile	Arg	Asp	Lys	Ser	Thr	Tyr	
			100						105					110			
	Ala	Pro	Ser	Arg	Ala	Thr	Tyr	Lys	Val	Tyr	Ile	Ile	Asp	Glu	Val	His	
25			115					120					125				
	Met	Leu	Ser	Thr	Gly	Ala	Phe	Asn	Ala	Leu	Leu	Lys	Thr	Leu	Glu	Glu	
		130					135					140					
	Pro	Thr	Glu	Asn	Val	Phe	Ile	Leu	Ala	Thr	Thr	Glu	Leu	His	Lys	Ile	
30	145					150					155					160	
	Pro	Ala	Thr	Ile	Leu	Ser	Arg	Val	Gln	Arg	Phe	Glu	Phe	Lys	Ala	Ile	
					165					170					175		
35	Lys	Gln	Lys	Ala	Ile	Arg	Glu	His	Leu	Ala	Trp	Val	Leu	Asp	Lys	Glu	
				180					185					190			
	Gly	Ile	Ala	Tyr	Glu	Val	Asp	Ala	Leu	Asn	Leu	Ile	Ala	Arg	Arg	Ala	
40			195					200					205				
	Glu	Gly	Gly	Met	Arg	Asp	Ala	Leu	Ser	Ile	Leu	Asp	Gln	Ala	Leu	Ser	
		210					215					220					
	Leu	Ser	Pro	Asp	Asn	Gln	Val	Ala	Ile	Ala	Ile	Ala	Glu	Glu	Ile	Thr	
45	225					230				235						240	
	Gly	Ser	Ile	Ser	Ile	Leu	Ala	Leu	Gly	Asp	Tyr	Val	Arg	Tyr	Val	Ser	
					245					250					255		
50	Gln	Glu	Gln	Ala	Thr	Gln	Ala	Leu	Ala	Ala	Leu	Glu	Thr	Ile	Tyr	Asp	
				260				265						270			
	Ser	Gly	Lys	Ser	Met	Ser	Arg	Phe	Ala	Thr	Asp	Leu	Leu	Thr	Tyr	Leu	
55			275					280					285				
	Arg	Asp	Leu	Leu	Val	Val	Lys	Ala	Gly	Gly	Asp	Asn	Gln	Arg	Gln	Ser	
		290					295					300					
	Ala	Val	Phe	Asp	Thr	Asn	Leu	Ser	Leu	Ser	Ile	Asp	Arg	Ile	Phe	Gln	
60	305					310					315					320	
	Met	Ile	Thr	Val	Val	Thr	Ser	His	Leu	Pro	Glu	Ile	Lys	Lys	Gly	Thr	
					325					330					335		

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	His	Pro	Arg	Ile	Tyr	Ala	Glu	Met	Met	Thr	Ile	Gln	Leu	Ala	Gln	Lys
				340					345					350		
5	Glu	Gln	Ile	Leu	Ser	Gln	Val	Asn	Leu	Ser	Gly	Glu	Leu	Ile	Ser	Glu
			355					360					365			
	Ile	Glu	Thr	Leu	Lys	Asn	Glu	Leu	Ala	Gln	Leu	Lys	Gln	Gln	Leu	Ser
10		370					375					380				
	Gln	Leu	Gln	Ser	Arg	Pro	Asp	Ser	Leu	Ala	Arg	Ser	Asp	Lys	Thr	Lys
	385					390					395					400
15	Pro	Lys	Thr	Thr	Ser	Tyr	Arg	Val	Asp	Arg	Val	Thr	Ile	Leu	Lys	Ile
					405					410					415	
	Met	Glu	Glu	Thr	Val	Arg	Asn	Ser	Gln	Gln	Ser	Arg	Gln	Tyr	Leu	Asp
				420					425					430		
20	Ala	Leu	Lys	Asn	Ala	Trp	Asn	Glu	Ile	Leu	Asp	Asn	Ile	Ser	Ala	Gln
			435					440					445			
	Asp	Arg	Ala	Leu	Leu	Met	Gly	Ser	Glu	Pro	Val	Leu	Ala	Asn	Ser	Glu
25		450					455					460				
	Asn	Ala	Ile	Leu	Ala	Phe	Glu	Ala	Ala	Phe	Asn	Ala	Glu	Gln	Val	Met
	465					470					475					480
30	Ser	Arg	Asn	Asn	Leu	Asn	Asp	Met	Phe	Gly	Asn	Ile	Met	Ser	Lys	Ala
					485					490					495	
	Ala	Gly	Phe	Ser	Pro	Asn	Ile	Leu	Ala	Val	Pro	Arg	Thr	Asp	Phe	Gln
				500					505					510		
35	His	Ile	Arg	Lys	Glu	Phe	Ala	Gln	Gln	Met	Lys	Ser	Gln	Lys	Asp	Ser
			515					520						525		
	Val	Gln	Glu	Glu	Gln	Glu	Val	Ala	Leu	Asp	Ile	Pro	Glu	Gly	Phe	Asp
40		530					535					540				
	Phe	Leu	Leu	Asp	Lys	Ile	Asn	Thr	Ile	Asp	Asp					
	545					550				555						

45 The present invention also relates to the *dnaN* gene of *Streptococcus pyogenes* encoding the β subunit. The *dnaN* gene has a nucleotide sequence which corresponds to SEQ. ID. No. 27 as follows:

50	atgattcaat	tttcaattaa	tcgcacatta	tttattcatg	ctttaaatac	aactaaacgt	60
	gctattagca	ctaaaaatgc	cattcctatt	ctttcatcaa	taaaaattga	agtcacttct	120
	acaggagtaa	ctttaacagg	gtctaacggt	caaatatcaa	ttgaaaacac	tattcctgta	180
	agtaatgaaa	atgctggttt	gctaattacc	tctccaggag	ctattttatt	agaagctagt	240
	ttttttatta	atattatttc	aagtttgcca	gatattagta	taaatgtaa	agaaattgaa	300
	caacaccaag	ttgttttaac	cagtggtaaa	tcagagatta	ccttaaaagg	aaaagatgtt	360
	gaccagtatc	ctcgtctaca	agaagtatca	acagaaaatc	ctttgatttt	aaaaacaaaa	420
55	ttattgaagt	ctattattgc	tgaacagct	tttgcagcca	gtttacaaga	aagtcgtcct	480
	attttaacag	gagttcatat	tgtattaagt	aatcataaag	attttaaagc	agtagcgact	540
	gactctcatc	gtatgagcca	acgtttaatc	actttggaca	atacttcagc	agatttgatg	600
	gtagttcttc	caagtaaadc	tttgagagaa	ttttcagcag	tatttacaga	tgatattgag	660
	accgttgagg	tatttttttc	accaagccaa	atcttggtca	gaagtgaaca	catttctttt	720

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5 tatacacgcc tcttagaagg aaattatccc gatacagacc gtttattaat gacagaattt 780
gagacggagg ttgttttcaa tacccaatcc cttcgccacg ctatggaacg tgccttcttg 840
atttctaattg ctactcaaaa tggtagtggtt aagcttgaga ttactcaaaa tcatatttca 900
gctcatgtta actcacctga gggttggaag gtaaacgagg atttagatat tgtagtcag 960
tctggtagtg atttaactat cagcttcaat ccaacttacc ttattgagtc tttaaaagct 1020
attaaaagtg aaacagtaaa aattcatttc ttatcaccag ttcgaccatt caccctaaca 1080
ccaggcgatg aggaagaaag ttttatccaa ttaattacac cagtagaac aaac 1134

The encoded β subunit has an amino acid sequence corresponding to SEQ. ID. No. 28
as follows:

10 Met Ile Gln Phe Ser Ile Asn Arg Thr Leu Phe Ile His Ala Leu Asn
1 5 10 15
15 Thr Thr Lys Arg Ala Ile Ser Thr Lys Asn Ala Ile Pro Ile Leu Ser
20 25 30
Ser Ile Lys Ile Glu Val Thr Ser Thr Gly Val Thr Leu Thr Gly Ser
35 40 45
20 Asn Gly Gln Ile Ser Ile Glu Asn Thr Ile Pro Val Ser Asn Glu Asn
50 55 60
25 Ala Gly Leu Leu Ile Thr Ser Pro Gly Ala Ile Leu Leu Glu Ala Ser
65 70 75 80
Phe Phe Ile Asn Ile Ile Ser Ser Leu Pro Asp Ile Ser Ile Asn Val
85 90 95
30 Lys Glu Ile Glu Gln His Gln Val Val Leu Thr Ser Gly Lys Ser Glu
100 105 110
Ile Thr Leu Lys Gly Lys Asp Val Asp Gln Tyr Pro Arg Leu Gln Glu
115 120 125
35 Val Ser Thr Glu Asn Pro Leu Ile Leu Lys Thr Lys Leu Leu Lys Ser
130 135 140
Ile Ile Ala Glu Thr Ala Phe Ala Ala Ser Leu Gln Glu Ser Arg Pro
145 150 155 160
Ile Leu Thr Gly Val His Ile Val Leu Ser Asn His Lys Asp Phe Lys
165 170 175
45 Ala Val Ala Thr Asp Ser His Arg Met Ser Gln Arg Leu Ile Thr Leu
180 185 190
Asp Asn Thr Ser Ala Asp Leu Met Val Val Leu Pro Ser Lys Ser Leu
195 200 205
50 Arg Glu Phe Ser Ala Val Phe Thr Asp Asp Ile Glu Thr Val Glu Val
210 215 220
Phe Phe Ser Pro Ser Gln Ile Leu Phe Arg Ser Glu His Ile Ser Phe
225 230 235 240
Tyr Thr Arg Leu Leu Glu Gly Asn Tyr Pro Asp Thr Asp Arg Leu Leu
245 250 255
60 Met Thr Glu Phe Glu Thr Glu Val Val Phe Asn Thr Gln Ser Leu Arg
260 265 270

His Ala Met Glu Arg Ala Phe Leu Ile Ser Asn Ala Thr Gln Asn Gly
 275 280 285
 5 Thr Val Lys Leu Glu Ile Thr Gln Asn His Ile Ser Ala His Val Asn
 290 295 300
 Ser Pro Glu Val Gly Lys Val Asn Glu Asp Leu Asp Ile Val Ser Gln
 305 310 315 320
 10 Ser Gly Ser Asp Leu Thr Ile Ser Phe Asn Pro Thr Tyr Leu Ile Glu
 325 330 335
 Ser Leu Lys Ala Ile Lys Ser Glu Thr Val Lys Ile His Phe Leu Ser
 340 345 350
 15 Pro Val Arg Pro Phe Thr Leu Thr Pro Gly Asp Glu Glu Glu Ser Phe
 355 360 365
 20 Ile Gln Leu Ile Thr Pro Val Arg Thr Asn
 370 375

The present invention also relates to the *ssb* gene of *Streptococcus*
pyogenes encoding the single strand-binding protein (SSB). The *ssb* gene has a
 25 nucleotide sequence which corresponds to SEQ. ID. No. 29 as follows:

atgattaata atgtagtact agttgggtcgc atgaccaagg atgcagaact tcgttacaca 60
 ccaagtcaag tagctgtggc taccttcaca cttgctgtta accgtacctt taaaagccaa 120
 30 aatgggtgaac gcgaggcaga ttctattaac tgtgtgatct ggcgtcaacc ggctgaaaat 180
 ttagcgaact gggctaaaaa aggtgctttg atcggagtta cgggtcgtat tcatacacgt 240
 aactacgaaa accaacaagg acaacgtgtc tatgtaacag aagttggtgc agataatttc 300
 caaatgttgg aaagtcgtgc tacacgtgaa ggtggctcaa ctgggtcatt taatgggtggt 360
 tttacaata acacttcac atcaaacagt tactcagcgc ctgcacaaca aacgcctaac 420
 35 tttggaagag atgatagccc atttggaac tcaaaccgga tggatatctc agatgacgat 480
 ctccattct ag 492

The encoded SSB protein has an amino acid sequence corresponding to SEQ. ID.
 No. 30 as follows:

40 Met Ile Asn Asn Val Val Leu Val Gly Arg Met Thr Lys Asp Ala Glu
 1 5 10 15
 45 Leu Arg Tyr Thr Pro Ser Gln Val Ala Val Ala Thr Phe Thr Leu Ala
 20 25 30
 Val Asn Arg Thr Phe Lys Ser Gln Asn Gly Glu Arg Glu Ala Asp Phe
 35 40 45
 50 Ile Asn Cys Val Ile Trp Arg Gln Pro Ala Glu Asn Leu Ala Asn Trp
 50 55 60
 Ala Lys Lys Gly Ala Leu Ile Gly Val Thr Gly Arg Ile Gln Thr Arg
 65 70 75 80
 55 Asn Tyr Glu Asn Gln Gln Gly Gln Arg Val Tyr Val Thr Glu Val Val
 85 90 95

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Ala Asp Asn Phe Gln Met Leu Glu Ser Arg Ala Thr Arg Glu Gly Gly
100 105 110

5 Ser Thr Gly Ser Phe Asn Gly Gly Phe Asn Asn Asn Thr Ser Ser Ser
115 120 125

Asn Ser Tyr Ser Ala Pro Ala Gln Gln Thr Pro Asn Phe Gly Arg Asp
130 135 140

10 Asp Ser Pro Phe Gly Asn Ser Asn Pro Met Asp Ile Ser Asp Asp Asp
145 150 155 160

15 Leu Pro Phe

The present invention also relates to the *dnaG* gene of *Streptococcus pyogenes* encoding the primase. The *dnaG* gene has a nucleotide sequence which corresponds to SEQ. ID. No. 31 as follows:

20 atgggatttt tatggggagg tgacgatttg gcaattgaca aagaaatgat ttcccaagta 60
aaaaatagcg ttaatatattgt cgatgtcatt ggagaagtgg tcaaactttc ccgatcaggg 120
cggcattacc tcgggctttg cccattttcat aaggaaaaga caccctcttt taatgttgtt 180
25 gaagacagac aatttttttca ctgctttggc tgtggaaaat caggggatgt ttttaaattt 240
attgaggaat accgccaagt ccccttctta gaaagtgttc agattattgc cgataagact 300
ggtatgtcgc ttaatatacc gccaaagtcag gcagtacttg ctagccaaca caagcacct 360
aatcacgctt tgatgacact tcatgaggat gctgctaaat tttaccatgc agttttgatg 420
accactacca ttggtcaaga agctaggaag tacctttacc agagaggctt ggatgacca 480
ttaattgagc atttcaatat tggtttagcc ccagatgagt cagattatct ttatcaagct 540
30 ctttctaata aatacgagga aggtcaattg gttgcttcag gattgtttca cttgtccgat 600
caatccaata ccatttaaga cgcttttcga aatcgtatca tgtttccctt atcagatgac 660
cgagggcata ttattgcctt ttcaggacgt atctggacgg cagctgatat ggaaaagaga 720
caggcaaagt ataaaaattc aagaggaaca gttcttttta acaaattctta tgaattgtat 780
catctggaca aggcaaggcc tggtattgcc ctaaagcagt aagtgtttct aatggaagg 840
35 tttatggacg tgattgccgc ttaccgttcc ggctatgaaa atgctgttgc ttcaatgggg 900
acggcattga ctcaagaaca tgtcaatcac cttaagcaag tcaactaaaa agttgttttg 960
atztatgatg gtgacgatgc tggacaacat gctattgcaa aatcactaga attgcttaaa 1020
gattttgttg tcgaaattgt cagaatcccc aataaaatgg atcctgacga atttgtacaa 1080
cggcattccc cagaagcatt tgcagatttg cttaagcagt cacggatcag tagtgttgaa 1140
40 ttttttattg attacctaaa acctactaat gtagacaatt tgcaatcaca aattgtttat 1200
gtggagaaaa tggcaccatt gattgctcaa tcaccatcca tcacagetca acattcgtat 1260
attaacaaga ttgctgattt gttgccaaac tttgactatt ttcaagtaga acaatcagta 1320
aatgcattaa ggattcaaga taggcaaaaa catcaaggtc aaatagctca agccgtcagc 1380
aatcttgtga ccttaccatg gccaaaaagt ttgacagcta ttgctaagac agaaagtcac 1440
45 ctcatgcacg ggctcttaca tcatgactat ttattaaatg aatttcgaca tcgtgatgat 1500
ttttattttg atacctctac cttagaatta ctttatcaac ggctgaagca acaaggacac 1560
attacatctt atgatttgct agagatgtca gaggaagtta accgtgctta ttacaatggt 1620
ttagaagaaa accttcccaa agaagtagct cttggtgaga ttgatgatat tttatccaaa 1680
cgtgccaaac ttttagcaga gcgcgatctt cacaacaag ggaaaaaagt tagagaatct 1740
50 agtaacaaag gcgatcatca agcggtctta gaagtactag aacattttat tgcgcagaaa 1800
cgaaaaatgg aatag 1815

The encoded primase has an amino acid sequence corresponding to SEQ. ID. No. 32 as follows:

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	Met	Gly	Phe	Leu	Trp	Gly	Gly	Asp	Asp	Leu	Ala	Ile	Asp	Lys	Glu	Met	
	1				5					10					15		
5	Ile	Ser	Gln	Val	Lys	Asn	Ser	Val	Asn	Ile	Val	Asp	Val	Ile	Gly	Glu	
				20					25					30			
	Val	Val	Lys	Leu	Ser	Arg	Ser	Gly	Arg	His	Tyr	Leu	Gly	Leu	Cys	Pro	
			35					40					45				
10	Phe	His	Lys	Glu	Lys	Thr	Pro	Ser	Phe	Asn	Val	Val	Glu	Asp	Arg	Gln	
		50					55					60					
	Phe	Phe	His	Cys	Phe	Gly	Cys	Gly	Lys	Ser	Gly	Asp	Val	Phe	Lys	Phe	
15	65					70					75					80	
	Ile	Glu	Glu	Tyr	Arg	Gln	Val	Pro	Phe	Leu	Glu	Ser	Val	Gln	Ile	Ile	
					85					90					95		
20	Ala	Asp	Lys	Thr	Gly	Met	Ser	Leu	Asn	Ile	Pro	Pro	Ser	Gln	Ala	Val	
				100					105					110			
	Leu	Ala	Ser	Gln	His	Lys	His	Pro	Asn	His	Ala	Leu	Met	Thr	Leu	His	
			115					120					125				
25	Glu	Asp	Ala	Ala	Lys	Phe	Tyr	His	Ala	Val	Leu	Met	Thr	Thr	Thr	Ile	
		130					135					140					
	Gly	Gln	Glu	Ala	Arg	Lys	Tyr	Leu	Tyr	Gln	Arg	Gly	Leu	Asp	Asp	Gln	
30	145					150					155					160	
	Leu	Ile	Glu	His	Phe	Asn	Ile	Gly	Leu	Ala	Pro	Asp	Glu	Ser	Asp	Tyr	
					165					170					175		
35	Leu	Tyr	Gln	Ala	Leu	Ser	Lys	Lys	Tyr	Glu	Glu	Gly	Gln	Leu	Val	Ala	
				180					185					190			
	Ser	Gly	Leu	Phe	His	Leu	Ser	Asp	Gln	Ser	Asn	Thr	Ile	Tyr	Asp	Ala	
			195					200					205				
40	Phe	Arg	Asn	Arg	Ile	Met	Phe	Pro	Leu	Ser	Asp	Asp	Arg	Gly	His	Ile	
		210					215					220					
	Ile	Ala	Phe	Ser	Gly	Arg	Ile	Trp	Thr	Ala	Ala	Asp	Met	Glu	Lys	Arg	
45	225					230					235					240	
	Gln	Ala	Lys	Tyr	Lys	Asn	Ser	Arg	Gly	Thr	Val	Leu	Phe	Asn	Lys	Ser	
					245					250					255		
50	Tyr	Glu	Leu	Tyr	His	Leu	Asp	Lys	Ala	Arg	Pro	Val	Ile	Ala	Lys	Thr	
				260					265					270			
	His	Glu	Val	Phe	Leu	Met	Glu	Gly	Phe	Met	Asp	Val	Ile	Ala	Ala	Tyr	
			275					280					285				
55	Arg	Ser	Gly	Tyr	Glu	Asn	Ala	Val	Ala	Ser	Met	Gly	Thr	Ala	Leu	Thr	
		290					295					300					
	Gln	Glu	His	Val	Asn	His	Leu	Lys	Gln	Val	Thr	Lys	Lys	Val	Val	Leu	
60	305					310					315					320	
	Ile	Tyr	Asp	Gly	Asp	Asp	Ala	Gly	Gln	His	Ala	Ile	Ala	Lys	Ser	Leu	
					325					330					335		

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	Glu	Leu	Leu	Lys	Asp	Phe	Val	Val	Glu	Ile	Val	Arg	Ile	Pro	Asn	Lys	
				340					345					350			
5	Met	Asp	Pro	Asp	Glu	Phe	Val	Gln	Arg	His	Ser	Pro	Glu	Ala	Phe	Ala	
			355					360					365				
	Asp	Leu	Leu	Lys	Gln	Ser	Arg	Ile	Ser	Ser	Val	Glu	Phe	Phe	Ile	Asp	
			370				375					380					
10	Tyr	Leu	Lys	Pro	Thr	Asn	Val	Asp	Asn	Leu	Gln	Ser	Gln	Ile	Val	Tyr	
	385					390					395					400	
	Val	Glu	Lys	Met	Ala	Pro	Leu	Ile	Ala	Gln	Ser	Pro	Ser	Ile	Thr	Ala	
15				405						410					415		
	Gln	His	Ser	Tyr	Ile	Asn	Lys	Ile	Ala	Asp	Leu	Leu	Pro	Asn	Phe	Asp	
				420					425					430			
20	Tyr	Phe	Gln	Val	Glu	Gln	Ser	Val	Asn	Ala	Leu	Arg	Ile	Gln	Asp	Arg	
			435					440					445				
	Gln	Lys	His	Gln	Gly	Gln	Ile	Ala	Gln	Ala	Val	Ser	Asn	Leu	Val	Thr	
		450				455						460					
25	Leu	Pro	Met	Pro	Lys	Ser	Leu	Thr	Ala	Ile	Ala	Lys	Thr	Glu	Ser	His	
	465					470					475					480	
	Leu	Met	His	Arg	Leu	Leu	His	His	Asp	Tyr	Leu	Leu	Asn	Glu	Phe	Arg	
30					485					490					495		
	His	Arg	Asp	Asp	Phe	Tyr	Phe	Asp	Thr	Ser	Thr	Leu	Glu	Leu	Leu	Tyr	
				500					505					510			
35	Gln	Arg	Leu	Lys	Gln	Gln	Gly	His	Ile	Thr	Ser	Tyr	Asp	Leu	Ser	Glu	
			515				520						525				
	Met	Ser	Glu	Glu	Val	Asn	Arg	Ala	Tyr	Tyr	Asn	Val	Leu	Glu	Glu	Asn	
		530				535						540					
40	Leu	Pro	Lys	Glu	Val	Ala	Leu	Gly	Glu	Ile	Asp	Asp	Ile	Leu	Ser	Lys	
	545					550					555					560	
	Arg	Ala	Lys	Leu	Leu	Ala	Glu	Arg	Asp	Leu	His	Lys	Gln	Gly	Lys	Lys	
45					565					570					575		
	Val	Arg	Glu	Ser	Ser	Asn	Lys	Gly	Asp	His	Gln	Ala	Ala	Leu	Glu	Val	
				580				585						590			
50	Leu	Glu	His	Phe	Ile	Ala	Gln	Lys									
		595					600										

The present invention also relates to the *dnaB* gene of *Streptococcus pyogenes* encoding DnaB. The *dnaB* gene has a nucleotide sequence which corresponds to SEQ. ID. No. 33 as follows:

55

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atgaggttgc ctgaagtagc tgaattacga gttcaacccc aagatttact agcagagcaa 60
tctgttcttg ggtcaatctt tatctcacct gataagctga ttgcagtgag agaattttatc 120
agtccagacg atttttataa gtacgctcat aaaattatct ttcgggcaat gattaccctc 180
agcgatcgta atgatgccat tgatgcaacc actataagaa caatcctaga tgatcaagat 240

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5 gatctgcaaa gtattggtgg cttatcctat attgttgaac tagttaatag tgtcccaact 300
 agtgctaattg cagaatatta tgctaaaatt gtagctgaga aagctatgtt gcgtgatatt 360
 attgctaggt tgacagaatc tgtcaaccta gcttatgatg aaatttttaa accagaagag 420
 gttatcgctg gagttgagag agctttaatt gaactcaatg aacatagtaa tcgtagtggg 480
 tttcgcaaaa tttcagatgt gctaaaagtt aattacgagg ctttagaagc acgttctaag 540
 cagacttcaa atgttacagg tttaccaact gggttttagag accttgacaa gattacaaca 600
 gggtttacacc cagatcaatt agttatttta gctgctcggc cagcagtggg gaagactgcc 660
 tttgttctta atattgcgca aaatgtgggg actaagcaaa aaaagactgt tgctattttt 720
 10 tctttggaat tggttgctga aagtttagta gatcgatgc ttgcagcaga aggaatgggt 780
 gattcgacac gtttaagaac agggcaactc acagatcagg attggaataa tgtaacaatt 840
 gatattcggt aatcaggatc tattgagcag gatgcgata ttgtagcctt gattaaaatt 900
 actgaaatcc gcgcaagatc acggaaattg tctcaagaag tggatggtgg ttttaggtctc 960
 attgtaattg actacttaca gttgattaca ggaactaaac ccgaaaatcg tcagcaagag 1020
 15 gtttcagata tttcaagaca gcttaaaatc ctatgctaaag aattgaaagt accagttatt 1080
 gccctaagtc agctttctcg tggcggttag caaaggcaag ataaacgacc agttttatca 1140
 gatattcggt aatcaggatc tattgagcag gatgcgata ttgtagcctt cttataccgg 1200
 gacgattatt accgtaaaga atgtgatgat gctgaagaag ctgttgaaga taacacaatt 1260
 gaagttatcc tcgagaaaaa tagagctggg gcgcgtggaa cagtcaaact gatgttccaa 1320
 20 aaagaataca acaaattctc aagtatagcc cagtttgaag aaagataa 1368

The encoded DnaB has an amino acid sequence corresponding to SEQ. ID. No. 34 as follows:

25 Met Arg Leu Pro Glu Val Ala Glu Leu Arg Val Gln Pro Gln Asp Leu
 1 5 10 15
 Leu Ala Glu Gln Ser Val Leu Gly Ser Ile Phe Ile Ser Pro Asp Lys
 20 25 30
 30 Leu Ile Ala Val Arg Glu Phe Ile Ser Pro Asp Asp Phe Tyr Lys Tyr
 35 40 45
 Ala His Lys Ile Ile Phe Arg Ala Met Ile Thr Leu Ser Asp Arg Asn
 50 55 60
 35 Asp Ala Ile Asp Ala Thr Thr Ile Arg Thr Ile Leu Asp Asp Gln Asp
 65 70 75 80
 40 Asp Leu Gln Ser Ile Gly Gly Leu Ser Tyr Ile Val Glu Leu Val Asn
 85 90 95
 Ser Val Pro Thr Ser Ala Asn Ala Glu Tyr Tyr Ala Lys Ile Val Ala
 100 105 110
 45 Glu Lys Ala Met Leu Arg Asp Ile Ile Ala Arg Leu Thr Glu Ser Val
 115 120 125
 Asn Leu Ala Tyr Asp Glu Ile Leu Lys Pro Glu Glu Val Ile Ala Gly
 130 135 140
 50 Val Glu Arg Ala Gln Gly Ala Leu Ala Glu Ala Pro Ile Tyr Ile Asp
 145 150 155 160
 Asp Thr Pro Gly Ile Lys Ile Ala Leu Ile Glu Leu Asn Glu His Ser
 165 170 175
 55 Asn Arg Ser Gly Phe Arg Lys Ile Ser Asp Val Leu Lys Val Asn Tyr
 180 185 190
 60 Glu Ala Leu Glu Ala Arg Ser Lys Gln Thr Ser Asn Val Thr Gly Leu
 195 200 205

	Pro	Thr	Gly	Phe	Arg	Asp	Leu	Asp	Lys	Ile	Thr	Thr	Gly	Leu	His	Pro
	210						215					220				
5	Asp	Gln	Leu	Val	Ile	Leu	Ala	Ala	Arg	Pro	Ala	Val	Gly	Lys	Thr	Ala
	225					230					235					240
	Phe	Val	Leu	Asn	Ile	Ala	Gln	Asn	Val	Gly	Thr	Lys	Gln	Lys	Lys	Thr
					245					250					255	
10	Val	Ala	Ile	Phe	Ser	Leu	Glu	Met	Gly	Ala	Glu	Ser	Leu	Val	Asp	Arg
				260					265					270		
	Met	Leu	Ala	Ala	Glu	Gly	Met	Val	Asp	Ser	His	Ser	Leu	Arg	Thr	Gly
			275					280					285			
	Gln	Leu	Thr	Asp	Gln	Asp	Trp	Asn	Asn	Val	Thr	Ile	Thr	Glu	Ile	Arg
	290						295					300				
20	Ala	Arg	Ser	Arg	Lys	Leu	Ser	Gln	Glu	Val	Asp	Gly	Gly	Leu	Gly	Leu
	305					310					315					320
	Ile	Val	Ile	Asp	Tyr	Leu	Gln	Leu	Ile	Thr	Gly	Thr	Lys	Pro	Glu	Asn
					325					330					335	
25	Arg	Gln	Gln	Glu	Val	Ser	Asp	Ile	Ser	Arg	Gln	Leu	Lys	Ile	Leu	Ala
				340					345					350		
	Lys	Glu	Leu	Lys	Val	Pro	Val	Ile	Ala	Leu	Ser	Gln	Leu	Ser	Arg	Gly
			355					360					365			
	Val	Glu	Gln	Arg	Gln	Asp	Lys	Arg	Pro	Val	Leu	Ser	Asp	Ile	Arg	Glu
	370						375					380				
35	Ser	Gly	Ser	Ile	Glu	Gln	Asp	Ala	Asp	Ile	Val	Ala	Phe	Leu	Tyr	Arg
	385					390					395					400
	Asp	Asp	Tyr	Tyr	Arg	Lys	Glu	Cys	Asp	Asp	Ala	Glu	Glu	Ala	Val	Glu
					405					410					415	
40	Asp	Asn	Thr	Ile	Glu	Val	Ile	Leu	Glu	Lys	Asn	Arg	Ala	Gly	Ala	Arg
				420					425					430		
	Gly	Thr	Val	Lys	Leu	Met	Phe	Gln	Lys	Glu	Tyr	Asn	Lys	Phe	Ser	Ser
			435					440					445			
45	Ile	Ala	Gln	Phe	Glu	Glu	Arg									
	450						455									

50 Fragments of the above polypeptides or proteins are also encompassed
by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller

protein or peptide that can be tested for activity according to the procedures described below.

As an alternative, fragments of replication proteins can be produced by digestion of a full-length replication protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave replication proteins at different sites based on the amino acid sequence of the protein. Some of the fragments that result from proteolysis may be active and can be tested for activity as described below.

In another approach, based on knowledge of the primary structure of the protein, fragments of a replication protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences of replication proteins being produced. Alternatively, subjecting a full length replication protein to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which cotranslationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of at least about 20, more preferably at least about 30 to about 50, continuous bases of either SEQ. ID. Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33 under stringent conditions such as those characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of about 37°C and remaining bound when subject to washing the SSC buffer at a temperature of about 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of about 42°C and

remaining bound when subject to washing at about 42°C with 0.2x SSC buffer. Stringency conditions can be further varied by modifying the temperature and/or salt content of the buffer, or by modifying the length of the hybridization probe.

5 The proteins or polypeptides of the present invention are preferably produced in purified form (preferably at least 80%, more preferably 90%, pure) by conventional techniques. Typically, the proteins or polypeptides of the present invention is secreted into the growth medium of recombinant host cells. Alternatively, the proteins or polypeptides of the present invention are produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell
10 (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to purification procedures such as ammonium sulfate precipitation, gel filtration, ion exchange chromatography, FPLC, and HPLC.

15 The DNA molecule encoding replication polypeptides or proteins derived from Gram positive bacteria can be incorporated in cells using conventional recombinant DNA technology. Generally, this involved inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector
20 contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA
25 ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into
30 cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19,

pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called

the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the same codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably
5 promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote
10 transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter,
15 *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be
20 used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG
25 (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls. Additionally, the cell may carry the gene for a heterologous RNA polymerase such as from phage T7. Thus, a promoter specific for T7 RNA polymerase is used. The T7 RNA polymerase may be under inducible control.

30 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector,

which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, an SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding a replication polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, viruses, yeast, mammalian cells, insects, plants, and the like.

The invention provides efficient methods of identifying pharmacological agents or lead compounds for agents active at the level of a replication protein function, particularly DNA replication. Generally, these screening methods involve assaying for compounds which interfere with the replication activity. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Target therapeutic indications are limited only in that the target cellular function be subject to modulation, usually inhibition, by disruption of a replication activity or the formation of a complex comprising a replication protein and one or more natural intracellular binding targets. Target indications may include arresting cell growth or causing cell death resulting in recovery from the bacterial infection in animal studies.

A wide variety of assays for activity and binding agents are provided, including DNA synthesis, ATPase, clamp loading onto DNA, protein-protein binding assays, immunoassays, cell based assays, etc. The replication protein compositions, used to identify pharmacological agents, are in isolated, partially pure or pure form

and are typically recombinantly produced. The replication protein may be part of a fusion product with another peptide or polypeptide (e.g., a polypeptide that is capable of providing or enhancing protein-protein binding, stability under assay conditions (e.g., a tag for detection or anchoring), etc.). The assay mixtures comprise a natural
5 intracellular replication protein binding target such as DNA, another protein, NTP, or dNTP. For binding assays, while native binding targets may be used, it is frequently preferred to use portions (e.g., peptides, nucleic acid fragments) thereof so long as the portion provides binding affinity and avidity to the subject replication protein conveniently measurable in the assay. The assay mixture also comprises a candidate
10 pharmacological agent. Generally, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control (i.e., at zero concentration or below the limits of assay detection). Additional controls are often present such as a positive control, a dose response curve, use of known
15 inhibitors, use of control heterologous proteins, etc. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably they are small organic compounds and are obtained from a wide variety of sources, including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral
20 proteins (e.g., albumin, detergents, etc.), which may be used to facilitate optimal binding and/or reduce nonspecific or background interactions, etc. Also reagents that otherwise improve the efficiency of the assay (e.g., protease inhibitors, nuclease inhibitors, antimicrobial agents, etc.) may be used.

The invention provides replication protein specific assays and the
25 binding agents including natural intracellular binding targets such as other replication proteins, etc., and methods of identifying and making such agents, and their use in a variety of diagnostic and therapeutic applications, especially where disease is associated with excessive cell growth. Novel replication protein-specific binding agents include replication protein-specific antibodies and other natural intracellular
30 binding agents identified with assays such as one- and two-hybrid screens, non-natural intracellular binding agents identified in screens of chemical libraries, etc.

Generally, replication protein-specificity of the binding agent is shown by binding equilibrium constants. Such agents are capable of selectively binding a

replication protein (i.e., with an equilibrium constant at least about 10^7 M^{-1} , preferably, at least about 10^8 M^{-1} , more preferably, at least about 10^9 M^{-1}). A wide variety of cell-based and cell-free assays may be used to demonstrate replication protein-specific activity, binding, gel shift assays, immunoassays, etc.

5 The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the replication protein specifically binds the cellular binding target, portion, or analog. The mixture of components can be added in any order that provides for the requisite bindings. Incubations may be performed at any temperature which facilitates optimal binding, typically between 4°C and 40°C , more commonly between 15°C and 40°C . Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening, and are typically between 0.1 and 10 hours, preferably less than 5 hours, more preferably less than 2 hours.

10 After incubation, the presence or absence of activity or specific binding between the replication protein and one or more binding targets is detected by any convenient way. For cell-free activity and binding type assays, a separation step may be used to separate the activity product or the bound from unbound components. Separation may be effected by precipitation (e.g., immunoprecipitation), immobilization (e.g., on a solid substrate such as a microtiter plate), etc., followed by washing. Many assays that do not require separation are also possible such as use of europium conjugation in proximity assays or a detection system that is dependent on a product or loss of substrate.

15 Detection may be effected in any convenient way. For cell-free activity and binding assays, one of the components usually comprises or is coupled to a label. 25 A wide variety of labels may be employed – essentially any label that provides for detection of DNA product, loss of DNA substrate, conversion of a nucleotide substrate, or bound protein is useful. The label may provide for direct detection such as radioactivity, fluorescence, luminescence, optical, or electron density, etc. or indirect detection such as an epitope tag, an enzyme, etc. The label may be appended 30 to the protein (e.g., a phosphate group comprising a radioactive isotope of phosphorous), or incorporated into the DNA substrate or the protein structure (e.g., a methionine residue comprising a radioactive isotope of sulfur.) A variety of methods may be used to detect the label depending on the nature of the label and other assay

components. For example, the label may be detected bound to the solid substrate, or a portion of the bound complex containing the label may be separated from the solid substrate, and thereafter the label detected. Labels may be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfer, fluorescence emission, etc. or indirectly detected with antibody conjugates, etc. For example, in the case of radioactive labels, emissions may be detected directly (e.g., with particle counters) or indirectly (e.g., with scintillation cocktails and counters).

The present invention identifies the set of proteins that together result in a three component polymerase from bacteria that are distantly related to *E. coli*, such as Gram positive bacteria. Specifically, these bacteria lack several genes that *E. coli* DNA polymerase III has, such as *holD*, *holD* or *holE*. Further, *dnaX* is believed to encode only one protein, tau. Also, *holA* is quite divergent in homology suggesting it may function in another process in these organisms. Gram positive cells even have replication genes that *E. coli* does not, implying that they may not utilize the replication strategies exemplified by *E. coli*.

The present invention identifies genes and proteins that form a three component polymerase in Gram positive organisms, such as *S. pyogenes* and *S. aureus*. In *S. pyogenes* and *S. aureus*, the polymerase α -large, functions with a β clamp and a clamp loader component of $\tau\delta\delta'$. They display high speed and processivity in synthesis of ssDNA coated with SSB and primed with a DNA oligonucleotide.

This invention also expresses and purifies a protein from a Gram positive bacteria that is homologous to the *E. coli* beta subunit. The invention demonstrates that it behaves like a circular protein. Further, this invention shows that a beta subunit from a Gram positive bacteria is functional with both Pol III-L (α -large) from a Gram positive bacteria and with DNA polymerase III from a Gram negative bacteria. This result can be explained by an interaction between the clamp and the polymerase that has been conserved during the evolutionary divergence of Gram positive and Gram negative cells. A chemical inhibitor that would disrupt this interaction would be predicted to have a broad spectrum of antibiotic activity, shutting down replication in gram negative and gram positive cells alike. This assay, and others based on this interaction, can be devised to screen chemicals for such inhibition. Further, since all the proteins in this assay are highly overexpressed

through recombinant techniques, sufficient quantities of the protein reagents can be obtained for screening hundreds of thousands of compounds.

This invention also shows that the DnaE polymerase (α -small), encoded by the *dnaE* gene, functions with the beta clamp and $\tau\delta\delta'$ complex. The speed of DnaE is not significantly increased by $\tau\delta\delta'$ and β , but the processivity of DnaE is greatly increased by $\tau\delta\delta'$ and β . Hence, the DnaE polymerase, coupled with its β clamp on DNA (loaded by $\tau\delta\delta'$) may also be an important target for a candidate pharmaceutical drug.

The present invention provides methods by which replication proteins from a Gram positive bacteria are used to discover new pharmaceutical agents. The function of replication proteins is quantified in the presence of different chemical compounds. A chemical compound that inhibits the function is a candidate antibiotic. Some replication proteins from a Gram positive bacteria and from a Gram negative bacteria can be interchanged for one another. Hence, they can function as mixtures. Reactions that assay for the function of enzyme mixtures consisting of proteins from Gram positive bacteria and from Gram negative bacteria can also be used to discover drugs. Suitable *E. coli* replication proteins are the subunits of its Pol III holoenzyme which are described in U.S. Patent Nos. 5,583,026 and 5,668,004 to O'Donnell, which are hereby incorporated by reference.

The methods described herein to obtain genes, and the assays demonstrating activity behavior of *S. pyogenes* and *S. aureus* replication proteins are likely to generalize to all members of the *Streptococcus* and *Staphylococcus* genres, as well as to all Gram positive bacteria. Such assays are also likely to generalize to other cells besides Gram positive bacteria which also share features in common with *S. pyogenes* and *S. aureus* that are different from *E. coli* (i.e., lacking *holC*, *holD*, or *holE*; having a *dnaX* gene encoding a single protein; or having a weak homology to *holA* encoding delta).

The present invention describes a method of identifying compounds which inhibit the activity of a polymerase product of *polC* or *dnaE*. This method is carried out by forming a reaction mixture that includes a primed DNA molecule, a polymerase product of *polC* or *dnaE*, a candidate compound, a dNTP, and optionally either a beta subunit, a tau complex, or both the beta subunit and the tau complex, wherein at least one of the polymerase product of *polC* or *dnaE*, the beta subunit, the

tau complex, or a subunit or combination of subunits thereof is derived from a Eubacteria other than *Escherichia coli*; subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound; analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and identifying the candidate compound in the reaction mixture where there is an absence of nucleic acid polymerization extension products. Preferably, the polymerase product of *polC* or *dnaE*, the beta subunit, the tau complex, or the subunit or combination of subunits thereof is derived from a Gram positive bacterium, more preferably a *Streptococcus* bacterium such as *S. pyogenes* or a *Staphylococcus* bacterium such as *S. aureus*.

The present invention describes a method to identify chemicals that inhibit the activity of the three component polymerase. This method involves contacting primed DNA with the DNA polymerase in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions effective to achieve nucleic acid polymerization in the absence of the candidate pharmaceutical and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product.

The present invention describes a method to identify candidate pharmaceuticals that inhibit the activity of a clamp loader complex and a beta subunit in stimulating the DNA polymerase. The method includes contacting a primed DNA (which may be coated with SSB) with a DNA polymerase, a beta subunit, and a tau complex (or subunit or subassembly of the tau complex) in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions which, in the absence of the candidate pharmaceutical, would effect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The DNA polymerase, the beta subunit, and/or the tau complex or subunit(s) thereof are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit and a DNA polymerase to interact physically. This method involves contacting the beta subunit with the DNA polymerase in the presence

of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA polymerase and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta unit and the DNA polymerase. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the DNA polymerase. The DNA polymerase and/or the beta subunit are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a beta subunit and a tau complex (or a subunit or subassembly of the tau complex) to interact. This method includes contacting the beta subunit with the tau complex (or subunit or subassembly of the tau complex) in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the tau complex (or the subunit or subassembly of the tau complex) and the beta subunit would interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the beta subunit and the tau complex (or the subunit or subassembly of the tau complex). The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the tau complex (or the subunit or subassembly of the tau complex). The beta subunit and/or the tau complex or subunit thereof is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a tau complex (or a subassembly of the tau complex) to assemble a beta subunit onto a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) with the tau complex (or the subassembly thereof) and the beta subunit in the presence of the candidate pharmaceutical, and ATP or dATP to form a reaction mixture. The reaction mixture is subjected to conditions under which the tau complex (or subassembly) assembles the beta subunit on the DNA molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit and/or the tau complex are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the ability of a tau complex (or a subunit(s) of the tau complex) to disassemble a beta subunit from a DNA molecule. This method comprises contacting a DNA molecule onto which the beta subunit has been assembled in the presence of the candidate pharmaceutical, to form a reaction mixture. The reaction mixture is subjected to conditions under which the tau complex (or a subunit(s) or subassembly of the tau complex) disassembles the beta subunit from the DNA molecule absent the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the presence of the beta subunit on the DNA molecule. The beta subunit and/or the tau complex are derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that disassemble a beta subunit from a DNA molecule. This method involves contacting a circular primed DNA molecule (which may be coated with SSB) upon which the beta subunit has been assembled (e.g. by action of the tau complex) with the candidate pharmaceutical. The presence or absence of the beta subunit on the DNA molecule in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of the beta subunit on the DNA molecule. The beta subunit is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the dATP/ATP binding activity of a tau complex or a tau complex subunit (e.g. tau subunit). This method includes contacting the tau complex (or the tau complex subunit) with dATP/ATP either in the presence or absence of a DNA molecule and/or the beta subunit in the presence of the candidate pharmaceutical to form a reaction. The reaction mixture is subjected to conditions in which the tau complex (or the subunit of tau complex) interacts with dATP/ATP in the absence of the candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP is bound to the tau complex (or the subunit of tau complex) in the presence of the candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of hydrolysis. The tau complex and/or the beta subunit is derived from a Gram positive bacterium.

The present invention describes a method to identify chemicals that inhibit the dATP/ATPase activity of a tau complex or a tau complex subunit (e.g., the

tau subunit). This method involves contacting the tau complex (or the tau complex subunit) with dATP/ATP either in the presence or absence of a DNA molecule and/or a beta subunit in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions in which the tau subunit (or complex) hydrolyzes dATP/ATP in the absence of the candidate pharmaceutical. The reaction is analyzed to determine if dATP/ATP was hydrolyzed. Suitable candidate pharmaceuticals are identified by the absence of hydrolysis. The tau complex and/or the beta subunit is derived from a Gram positive bacterium.

Further methods for identifying chemicals that inhibit the activity of a DNA polymerase encoded by either the *dnaE* gene, *polC* gene, or their accessory proteins (i.e., clamp loader, clamp, etc.), are as follows:

1) Contacting a primed DNA molecule with the encoded product of the *dnaE* gene or *polC* gene in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid polymerization and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of extension product. The protein encoded by the *dnaE* gene and *PolC* gene is derived from a Gram positive bacterium.

2) Contacting a linear primed DNA molecule with a beta subunit and the encoded product of *dnaE* or *PolC* in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid polymerization, and the presence or absence of the extension product in the reaction mixture is analyzed. The candidate pharmaceutical is detected by the absence of extension product. The protein encoded by the *dnaE* gene and *PolC* gene is derived from a Gram positive bacterium.

3) Contacting a circular primed DNA molecule (may be coated with SSB) with a tau complex, a beta subunit and the encoded product of a *dnaE* gene or *PolC* gene in the presence of the candidate pharmaceutical, and dNTPs (or modified dNTPs) to form a reaction mixture. The reaction mixture is subjected to conditions, which in the absence of the candidate pharmaceutical, affect nucleic acid polymerization, and the presence or absence of the extension product in the reaction

mixture is analyzed. The candidate pharmaceutical is detected by the absence of product. The protein encoded by the *dnaE* gene and *PolC* gene, the beta subunit, and/or the tau complex are derived from a Gram positive bacterium.

4) Contacting a beta subunit with the product encoded by a *dnaE* gene or *PolC* gene in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is then analyzed for interaction between the beta subunit and the product encoded by the *dnaE* gene or *PolC* gene. The candidate pharmaceutical is detected by the absence of interaction between the beta subunit and the product encoded by the *dnaE* gene or *PolC* gene. The beta subunit and/or the protein encoded by the *dnaE* gene and *PolC* gene is derived from a Gram positive bacterium.

5) The present invention discloses a method to identify chemicals that inhibit a DnaB helicase. The method includes contacting the DnaB helicase with a DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support helicase activity in the absence of the candidate pharmaceutical. The DNA duplex molecule in the reaction mixture is analyzed for whether it is converted to ssDNA. The candidate pharmaceutical is detected by the absence of conversion of the duplex DNA molecule to the ssDNA molecule. The DnaB helicase is derived from a Gram positive bacterium.

6) The present invention describes a method to identify chemicals that inhibit the nucleoside or deoxynucleoside triphosphatase activity of a DnaB helicase. The method includes contacting the DnaB helicase with a DNA molecule substrate that has a duplex region in the presence of a nucleoside or deoxynucleoside triphosphate energy source and the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support nucleoside or deoxynucleoside triphosphatase activity of the DnaB helicase in the absence of the candidate pharmaceutical. The candidate pharmaceutical is detected by the absence of conversion of nucleoside or deoxynucleoside triphosphate to nucleoside or deoxynucleoside diphosphate. The DnaB helicase is derived from a Gram positive bacterium.

7) The present invention describes a method to identify chemicals that inhibit a primase. The method includes contacting primase with a ssDNA molecule in the presence of a candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions that support primase activity (e.g., the presence of nucleoside or deoxynucleoside triphosphates, appropriate buffer, presence or absence of DnaB helicase) in the absence of the candidate pharmaceutical. Suitable candidate pharmaceuticals are identified by the absence of primer formation detected either directly or indirectly. The primase is derived from a Gram positive bacterium.

8) The present invention describes a method to identify chemicals that inhibit the ability of a primase and the protein encoded by a *dnaB* gene to interact. This method includes contacting the primase with the protein encoded by the *dnaB* gene in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the primase and the protein encoded by the *dnaB* gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the primase and the protein encoded by the *dnaB* gene. The candidate pharmaceutical is detected by the absence of interaction between the primase and the protein encoded by the *dnaB* gene. The primase and/or the *dnaB* gene are derived from a Gram positive bacterium.

9) The present invention describes a method to identify chemicals that inhibit the ability of a protein encoded by a *dnaB* gene to interact with a DNA molecule. This method includes contacting the protein encoded by the *dnaB* gene with the DNA molecule in the presence of the candidate pharmaceutical to form a reaction mixture. The reaction mixture is subjected to conditions under which the DNA molecule and the protein encoded by the *dnaB* gene interact in the absence of the candidate pharmaceutical. The reaction mixture is then analyzed for interaction between the protein encoded by the *dnaB* gene and the DNA molecule. The candidate pharmaceutical is detected by the absence of interaction between the DNA molecule and the protein encoded by the *dnaB* gene. The *dnaB* gene is derived from a Gram positive bacterium.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention, but they are by no means intended to limit its scope.

5

Example 1 - Materials

Labeled deoxy- and ribonucleoside triphosphates were from Dupont-New England Nuclear; unlabelled deoxy- and ribonucleoside triphosphates were from Pharmacia-LKB; *E. coli* replication proteins were purified as described, alpha, epsilon, gamma, and tau (Studwell et al., "Processive Replication is Contingent on the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol. Chem., 265:1171-1178 (1990), which is hereby incorporated by reference), beta (Kong et al., "Three Dimensional Structure of the Beta Subunit of *Escherichia coli* DNA Polymerase III Holoenzyme: A Sliding DNA Clamp," Cell, 69:425-437 (1992), which is hereby incorporated by reference), delta and delta prime (Dong et al., "DNA Polymerase III Accessory Proteins. I. *HolA* and *holB* Encoding δ and δ' ," J. Biol. Chem., 268:11758-11765 (1993), which is hereby incorporated by reference), chi and psi (Xiao et al., "DNA Polymerase III Accessory Proteins. III. *HolC* and *holD* Encoding chi and psi," J. Biol. Chem., 268:11773-11778 (1993), which is hereby incorporated by reference), theta (Studwell-Vaughan et al., "DNA Polymerase III Accessory Proteins. V. Theta Encoded by *holE*," J. Biol. Chem., 268:11785-11791 (1993), which is hereby incorporated by reference), and SSB (Weiner et al., "The Deoxyribonucleic Acid Unwinding Protein of *Escherichia coli*," J. Biol. Chem., 250:1972-1980 (1975), which is hereby incorporated by reference). *E. coli* Pol III core and clamp loader complex (composed of subunits gamma, delta, delta prime, chi, and psi) were reconstituted as described in Onrust et al., "Assembly of a Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and Sliding Clamps in One Holoenzyme Particle. I. Organization of the Clamp Loader," J. Biol. Chem., 270:13348-13357 (1995), which is hereby incorporated by reference. Pol III* was reconstituted and purified as described in Onrust et al., "Assembly of a Chromosomal Replication Machine: Two DNA Polymerases, a Clamp Loader and Sliding Clamps in One Holoenzyme Particle. III. Interface Between Two Polymerases and the Clamp

Loader," J. Biol. Chem., 270:13366-13377 (1995), which is hereby incorporated by reference. Protein concentrations were quantitated by the Protein Assay (Bio-Rad) method using bovine serum albumin (BSA) as a standard. DNA oligonucleotides were synthesized by Oligos etc. Calf thymus DNA was from Sigma. Buffer A is 20 mM Tris-HCl (pH=7.5), 0.5 mM EDTA, 2 mM DTT, and 20% glycerol. Replication
5 buffer is 20 mM Tris-Cl (pH 7.5), 8 mM MgCl₂, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, 0.5 mM ATP, 3 mM each dCTP, dGTP, dATP, and 20 µM [α-³²P]dTTP. P-cell buffer is 50 mM potassium phosphate (pH 7.6), 5 mM DTT, 0.3 mM EDTA, 20% glycerol. T.E. buffer is 10 mM Tris-HCl (pH 7.5), 1 mM EDTA.
10 Cell lysis buffer is 50 mM Tris-HCl (pH 8.0) 10 % sucrose, 1 M NaCl, 0.3 mM spermidine.

Example 2 - Calf Thymus DNA Replication Assays

15 These assays were used in the purification of DNA polymerases from *S. aureus* cell extracts. Assays contained 2.5 µg activated calf thymus DNA in a final volume of 25 µl replication buffer. An aliquot of the fraction to be assayed was added to the assay mixture on ice followed by incubation at 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen et al., "Primase, the DnaG
20 Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference.

Example 3 - PolydA-oligodT Replication Assays

25 PolydA-oligodT was prepared as follows. PolydA of average length 4500 nucleotides was purchased from SuperTecs. OligodT35 was synthesized by Oligos etc. 145 µl of 5.2 mM (as nucleotide) polydA and 22 µl of 1.75 mM (as nucleotide) oligodT were mixed in a final volume of 2100 µl T.E. buffer (ratio as nucleotide was 21:1 polydA to oligodT). The mixture was heated to boiling in a 1 ml
30 eppendorf tube, then removed and allowed to cool to room temperature. Assays were performed in a final volume of 25 µl 20 mM Tris-Cl (pH 7.5), 8 mM MgCl₂, 5 mM DTT, 0.5 mM EDTA, 40 µg/ml BSA, 4% glycerol, containing 20 µM [α-³²P]dTTP

and 0.36 μ g polydA-oligodT. Proteins were added to the reaction on ice, then shifted to 37°C for 5 min. DNA synthesis was quantitated using DE81 paper as described in Rowen et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference.

Example 4 - Singly Primed M13mp18 ssDNA Replication Assays

M13mp18 was phenol extracted from phage and purified by two successive bandings (one downward and one upward) in cesium chloride gradients. M13mp18 ssDNA was singly primed with a DNA 30mer (map position 6817-6846) as described in Studwell et al. "Processive Replication is Contingent on the Exonuclease Subunit of DNA Polymerase III Holoenzyme," J. Biol. Chem., 265:1171-1178 (1990), which is hereby incorporated by reference. Replication assays contained 72 ng of singly primed M13mp18 ssDNA in a final volume of 25 μ l of replication buffer. Other proteins added to the assay, and their amounts, are indicated in the Brief Description of the Drawings. Reactions were incubated for 5 min. at 37°C and then were quenched upon adding an equal volume of 1% SDS and 40 mM EDTA. DNA synthesis was quantitated using DE81 paper as described in Rowen et al., "Primase, the DnaG Protein of *Escherichia coli*. An Enzyme Which Starts DNA Chains," J. Biol. Chem., 253:758-764 (1979), which is hereby incorporated by reference, and product analysis was performed in a 0.8% native agarose gel followed by autoradiography.

Example 5 - Genomic *Staphylococcus aureus* DNA

Two strains of *S. aureus* were used. For PCR of the first fragment of the *dnaX* gene sequence, the strain was ATCC 25923. For all other work the strain was strain 4220 (a gift of Dr. Pat Schlievert, University of Minnesota). This strain lacks a gene needed for producing toxic shock (Kreiswirth et al., "The Toxic Shock Syndrome Exotoxin Structural Gene is Not Detectably Transmitted by a Prophage," Nature, 305:709-712 (1996) and Balan et al., "Autocrine Regulation of Toxin Synthesis by *Staphylococcus aureus*," Proc. Natl. Acad. Sci. USA, 92:1619-1623

(1995), which are hereby incorporated by reference). *S. aureus* cells were grown overnight at 37°C in LB containing 0.5% glucose. Cells were collected by centrifugation (24 g wet weight). Cells were resuspended in 80 ml solution I (50 mM glucose, 10 mM EDTA, 25 mM Tris-HCL (pH 8.0)). SDS and NaOH were then added to 1% and 0.2 N, respectively, followed by incubation at 65°C for 30 min. to lyse the cells. 68.5 ml of 3 M sodium acetate (pH 5.0) was added followed by centrifugation at 12,000 rpm for 30 min. The supernatant was discarded and the pellet was washed twice with 50 ml of 6M urea, 10 mM Tris-HCL (pH 7.5), 1 mM EDTA using a dounce homogenizer. After each wash, the resuspended pellet was collected by centrifugation (12,000 rpm for 20 min.). After the second wash, the pellet was resuspended in 50 ml 10 mM T.E. buffer using a dounce homogenizer and then incubated for 30 min. at 65°C. The solution was centrifuged at 12,000 rpm for 20 min., and the viscous supernatant was collected. 43.46 g CsCl₂ was added to the 50 ml of supernatant (density between 1.395-1.398) and poured into two 35 ml quick seal ultracentrifuge tubes (tubes were completely filled using the same density of CsCl₂ in T.E.). To each tube was added 0.5 ml of a 10 mg/ml stock of ethidium bromide. Tubes were spun at 55,000 rpm for 18 h at 18°C in a Sorvall TV860 rotor. The band of genomic DNA was extracted using a syringe and needle. Ethidium bromide was removed using two butanol extractions and then dialyzed against 4 l of T.E. at pH 8.0 overnight. The DNA was recovered by ethanol precipitation and then resuspended in T.E. buffer (1.7 mg total) and stored at -20°C.

Example 6 - Cloning and Purification of *S. aureus* Pol III-L

To further characterize the mechanism of DNA replication in *S. aureus*, large amounts of its replication proteins were produced through use of the genes. The *polC* gene encoding *S. aureus* Pol III-L (alpha-large) subunit has been sequenced and expressed in *E. coli* (Pacitti et al., "Characterization and Overexpression of the Gene Encoding *Staphylococcus aureus* DNA Polymerase III," Gene, 165:51-56 (1995), which is hereby incorporated by reference). The previous work utilized a pBS[KS] vector for expression in which the *E. coli* RNA polymerase is used for gene transcription. In the earlier study, the *S. aureus polC* gene was precisely cloned at the 5' end encoding the N-terminus, but the amount of the gene

that remained past the 3' end was not disclosed and the procedure for subcloning the gene into the expression vector was only briefly summarized. Furthermore, the previous study does not show the level of expression of the *S. aureus* Pol III-L, nor the amount of *S. aureus* Pol III-L that is obtained from the induced cells. Since the previously published procedure could not be repeated and the efficiency of the expression vector could not be assessed, another strategy outlined below had to be developed.

The isolated *polC* gene was cloned into a vector that utilizes T7 RNA polymerase for transcription as this process generally expresses a large amount of protein. Hence, the *S. aureus polC* gene was cloned precisely into the start codon at the NdeI site downstream of the T7 promotor in a pET vector. As the *polC* gene contains an internal NdeI site, the entire gene could not be amplified and placed it into the NdeI site of a pET vector. Hence, a three step cloning strategy that yielded the desired clone was devised (Figure 1). These attempts were quite frustrating initially as no products of cloning in standard *E. coli* strains such as DH5 α , a typical laboratory strain for preparation of DNA, could be obtained. Finally, a cell that was mutated in several genes affecting DNA stability was useful in obtaining the desired products of cloning.

In brief, the cloning strategy required use of another expression vector (called pET1137kDa) in which the 37 kDa subunit of human RFC, the clamp loader of the human replication system, had been cloned into the pET11 vector. The gene encoding the 37kDa subunit contains an internal NsiI site, which was needed for the precise cloning of the isolated *polC* gene. This three step strategy is shown in Figure 1. In the first step, an approximately 2.3 kb section of the 5' section of the gene (encoding the N-terminus of Pol III-L) was amplified using the polymerase chain reaction (PCR). Primers were as follows:

Upstream (SEQ. ID. No. 35)

ggtggttaatt gtcttgcata tgacagagc

29

Downstream (SEQ. ID. No. 36)

agcgattaag tggattgccg gggtgtgatg c

31

Amplification was performed using 500 ng genomic DNA, 0.5 mM EDTA, 1 μ M of each primer, 1mM MgSO₄, 2 units vent DNA polymerase (New England Biolabs) in 100 μ l of vent buffer (New England Biolabs). Forty cycles were performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.; 72°C, 2.5 min. The product was digested with NdeI (underlined in the upstream primer) and NsiI (an internal site in the product) and the approximately 1.8 kb fragment was gel purified. A pET11 vector containing as an insert the 37 kDa subunit of human replication factor C (pET1137kDa) was digested with NdeI and NsiI and gel purified. The PCR fragment was ligated into the digested pET1137kDa vector and the ligation reaction was transformed into Epicurean coli supercompetent SURE 2 cells (Stratagene) and colonies were screened for the correct chimera (pET11PolC1) by examining minipreps for proper length and correct digestion products using NdeI and NsiI. In the second step, an approximately 2076 bp fragment containing the DNA encoding the C-terminus of Pol III-L subunit was amplified using the following sequences as primers:

Upstream (SEQ. ID. No. 37)

agcatcaca cccggcaatc cacttaatcg c 31

Downstream (SEQ. ID. No. 38)

gactacgcca tgggcattaa ataaataacc 29

The amplification cycling scheme was as described above except the elongation step at 72°C was for 2 min. The product was digested with BamHI (underlined in the downstream primer) and NsiI (internal to the product) and the approximately 480 bp product was gel purified and ligated into the pET11PolC1 that had been digested with NsiI/BamHI and gel purified (ligated product is pET11PolC2). To complete the expression vector, an approximately 2080 bp PCR product was amplified over the two NsiI sites internal to the gene using the following primers:

Upstream (SEQ. ID. No. 39)

gaagatgcat ataaacgtgc aagacctagt 30

Downstream (SEQ. ID. No. 40)

gtctgacgca cgaattgtaa agtaagatgc atag 34

The amplification cycling scheme was as described above except the 72°C elongation step was 2 min. The PCR product, and the pET11PolC2 vector, were digested with NsiI and gel purified. The ligation mixture was transformed as described above and colonies were screened for the correct chimera (pET11PolC).

To express Pol III-L polymerase, the pET11PolC plasmid was transformed into *E. coli* strain BL21(DE3). 24 L of *E. coli* BL21(DE3)pET11PolC were grown in LB media containing 50 µg/ml ampicillin at 37°C to an OD of 0.7 and then the temperature was lowered to 15°C. Cells were then induced for Pol III-L expression upon addition of 1 mM IPTG to produce the T7 RNA polymerase needed to transcribe *polC*. This step was followed by further incubation at 15°C for 18 h. Expression of *S. aureus* Pol III-L polymerase was so high that it could easily be visualized by Coomassie staining of a SDS polyacrylamide gel of whole cells (Figure 2A). The expressed protein migrated in the SDS polyacrylamide gel in a position expected for a 165 kDa polypeptide. In this procedure, it is important that cells are induced at 15°C, as induction at 37°C produces a truncated version of Pol III-L polymerase, of approximately 130 kDa.

Cells were collected by centrifugation at 5°C. Cells (12 g wet weight) were stored at -70°C. The following steps were performed at 4°C. Cells were thawed and lysed in cell lysis buffer as described (final volume = 50 ml) and were passed through a French Press (Amico) at a minimum of 20,000 psi. PMSF (2 mM) was added to the lysate as the lysate was collected from the French Press. DNA was removed and the lysate was clarified by centrifugation. The supernatant was dialyzed for 1 h against Buffer A containing 50 mM NaCl. The final conductivity was equivalent to 190 mM NaCl. Supernatant (24 ml, 208 mg) was diluted to 50 ml using Buffer A to bring the conductivity to 96 mM MgCl₂, and then was loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was eluted with a 160 ml linear gradient of Buffer A from 50 mM NaCl to 500 mM NaCl. Seventy five fractions (1.3 ml each) were collected (Figure 2B). Aliquots were analyzed for their ability to synthesize DNA, and 20 µl of each fraction was analyzed by Coomassie staining of an SDS polyacrylamide gel. Based on the DNA synthetic capability, and the correct size band in the gel, fractions 56-65 containing Pol III-L polymerase were pooled (22 ml, 31 mg). The pooled fractions were dialyzed

overnight at 4°C against 50 mM phosphate (pH 7.6), 5 mM DTT, 0.1 mM EDTA, 2 mM PMSF, and 20 % glycerol (P-cell buffer). The dialyzed pool was loaded onto a 4.5 ml phosphocellulose column equilibrated in P-cell buffer, and then eluted with a 25 ml linear gradient of P-cell buffer from 0 M NaCl to 0.5 M NaCl. Fractions of 1 ml were collected and analyzed in a SDS polyacrylamide gel stained with Coomassie Blue (Figure 2C). Fractions 20-36 contained the majority of the Pol III-large at a purity of greater than 90 % (5 mg).

Example 7 - *S. aureus* Pol III-L is Not Processive on its Own

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The Pol III-L polymerase purifies from *B. subtilis* as a single subunit without accessory factors (Barnes et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference). Hence, it seemed possible that it may be a Type I replicase (e.g., like T5 polymerase) and, thus, be capable of extending a single primer full length around a long singly primed template. To perform this experiment, a template M13mp18 ssDNA primed with a single DNA oligonucleotide either in the presence or absence of SSB was used. DNA products were analyzed in a neutral agarose gel which resolved products by size. The results showed that Pol III-L polymerase was incapable of extending the primer around the DNA (to form a completed duplex circle referred to as replicative form II ("RFII")) whether SSB was present or not. This experiment has been repeated using more enzyme and longer times, but no full length RFII products are produced. Hence, Pol III-L would appear not to follow the paradigm of the T5 system (Type I replicase) in which the polymerase is efficient in synthesis in the absence of any other protein(s).

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Example 8 - Cloning and Purification of *S. aureus* Beta Subunit

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The sequence of an *S. aureus* homolog of the *E. coli* *dnaN* gene (encoding the beta subunit) was obtained in a study in which the large *recF* region of DNA was sequenced (Alonso et al., "Nucleotide Sequence of the *recF* Gene Cluster From *Staphylococcus aureus* and Complementation Analysis in *Bacillus subtilis* *recF* Mutants," Mol. Gen. Genet., 246:680-686 (1995), Alonso et al., "Nucleotide

Sequence of the *recF* Gene Cluster From *Staphylococcus aureus* and
Complementation Analysis in *Bacillus subtilis recF* Mutants," Mol. Gen. Genet.,
248:635-636 (1995), which are hereby incorporated by reference). Sequence
alignment of the *S. aureus* beta and *E. coli* beta show approximately 30% identity.
5 Overall this level of homology is low and makes it uncertain that *S. aureus* beta will
have the same shape and function as the *E. coli* beta subunit.

To obtain *S. aureus* beta protein, the *dnaN* gene was isolated and
precisely cloned into a pET vector for expression in *E. coli*. *S. aureus* genomic DNA
was used as template to amplify the homolog of the *dnaN* gene (encoding the putative
10 beta). The upstream and downstream primers were designed to isolate the *dnaN* gene
by PCR amplification from genomic DNA. Primers were:

Upstream (SEQ. ID. No. 41)

cgactggaag gagttttaac atatgatgga attcac 36

Downstream (SEQ. ID. No. 42)

ttatatggat ccttagtaag ttctgattgg 30

The NdeI site used for cloning into pET16b (Novagen) is underlined in the Upstream
20 primer and the BamHI site used for cloning into pET16b is underlined in the
Downstream primer. The NdeI and BamHI sites were used for directional cloning
into pET16 (Figure 3). Amplification was performed using 500 ng genomic DNA, 0.5
mM dNTPs, 1 μ M of each primer, 1mM MgSO₄, 2 units vent DNA polymerase in 100
ul of vent buffer. Forty cycles were performed using the following cycling scheme:
25 94°C, 1 min; 60°C, 1 min.; 72°C, 1 min. 10s. The 1167 bp product was digested with
NdeI and BamHI and purified in a 0.7 % agarose gel. The pure digested fragment was
ligated into the pET16b vector which had been digested with NdeI and BamHI and gel
purified in a 0.7% agarose gel. Ligated products were transformed into *E. coli*
30 competent SURE II cells (Stratagene) and colonies were screened for the correct
chimera by examining minipreps for proper length and correct digestion products
using NdeI and BamHI.

24 L of BL21(DE3)pETbeta cells were grown in LB containing 50
 μ g/ml ampicillin at 37°C to an O.D. of 0.7, and, then, the temperature was lowered to

15°C. IPTG was added to a concentration of 2 mM and after a further 18 h at 15°C to induce expression of *S. aureus* beta (Figure 4A). It is interesting to note that the beta subunit, when induced at 37°C, was completely insoluble. However, induction of cells at 15°C provided strong expression of beta and, upon cell lysis, over 50% of the beta was present in the soluble fraction.

Cells were harvested by centrifugation (44 g wet weight) and stored at -70°C. The following steps were performed at 4°C. Cells (44 g wet weight) were thawed and resuspended in 45 ml 1X binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris HCl (final pH 7.5)) using a dounce homogenizer. Cells were lysed using a French Pressure cell (Aminco) at 20,000 psi, and then 4.5 ml of 10 % polyamine P (Sigma) was added. Cell debris and DNA was removed by centrifugation at 13,000 rpm for 30 min. at 4°C. The pET16beta vector places a 20 residue leader containing 10 histidine residues at the N-terminus of beta. Hence, upon lysing the cells, the *S. aureus* beta was greatly purified by chromatography on a nickel chelate resin (Figure 4B). The supernatant (890 mg protein) was applied to a 10 ml HiTrap Chelating Sepharose column (Pharmacia-LKB) equilibrated in binding buffer. The column was washed with binding buffer, then eluted with a 100 ml linear gradient of 60 mM imidazole to 1 M imidazole in binding buffer. Fractions of 1.35 ml were collected. Fractions were analyzed for the presence of beta in an SDS polyacrylamide gel stained with Coomassie Blue. Fractions 28-52, containing most of the beta subunit, were pooled (35 ml, 82 mg). Remaining contaminating protein was removed by chromatography on MonoQ. The *S. aureus* beta becomes insoluble as the ionic strength is lowered and, thus, the pool of beta was dialyzed overnight against Buffer A containing 400 mM NaCl. The dialyzed pool became slightly turbid indicating it was at its solubility limit at these concentrations of protein and NaCl. The insoluble material was removed by centrifugation (64 mg remaining) and, then, diluted 2-fold with Buffer A to bring the conductivity to 256. The protein was then applied to an 8 ml MonoQ column equilibrated in Buffer A plus 250 mM NaCl and then eluted with a 100 ml linear gradient of Buffer A from 0.25M NaCl to 0.75 M NaCl; fractions of 1.25 ml were collected (Figure 4C). Under these conditions, approximately 27 mg of the beta flowed through the column and the remainder eluted in fractions 1-18 (24 mg).

Example 9 - The *S. aureus* Beta Subunit Protein Stimulates *S. aureus* Pol III-L and *E. coli* Core

The experiment of Figure 5A, tests the ability of *S. aureus* beta to stimulate *S. aureus* Pol III-L on a linear polydA-oligodT template. Reactions are also performed with *E. coli* beta and Pol III core. The linear template was polydA of average length of 4500 nucleotides primed with a 30mer oligonucleotide of T residues. The first two lanes show the activity of Pol III-L either without (lane 1) or with *S. aureus* beta (lane 2). The result shows that the *S. aureus* beta stimulates Pol III-L approximately 5-6 fold. Lanes 5 and 6 show the corresponding experiment using *E. coli* core with (lane 6) or without (lane 5) *E. coli* beta. The core is stimulated over 10-fold by the *E. coli* beta subunit under the conditions used.

Although Gram positive and Gram negative cells diverged from one another long ago and components of one polymerase machinery would not be expected to be interchangeable, it was decided to test the activity of the *S. aureus* beta with *E. coli* Pol III core. Lanes 3 and 4 shows that the *S. aureus* beta also stimulates *E. coli* core about 5-fold. This result can be explained by an interaction between the clamp and the polymerase that has been conserved during the evolutionary divergence of gram positive and gram negative cells. A chemical inhibitor that would disrupt this interaction would be predicted to have a broad spectrum of antibiotic activity, shutting down replication in Gram negative and Gram positive cells alike. This assay, and others based on this interaction, can be devised to screen chemicals for such inhibition. Further, since all the proteins in this assay are highly overexpressed through recombinant techniques, sufficient quantities of the protein reagents can be obtained for screening hundreds of thousands of compounds.

In summary, the results show that *S. aureus* beta, produced in *E. coli*, is indeed an active protein (i.e., it stimulates polymerase activity). Furthermore, the results shows that Pol III-L functions with a second protein (i.e., *S. aureus* beta). Before this experiment, there was no assurance that Pol III-L, which is significantly different in structure from *E. coli* alpha, would function with another protein. For example, unlike *E. coli* alpha, which copurifies with several accessory proteins, Pol III-L purified from *B. subtilis* as a single protein with no other subunits attached (Barnes et al., "Purification of DNA Polymerase III of Gram-positive Bacteria," Methods in Enzy., 262:35-42 (1995), which is hereby incorporated by reference).

Finally, if one were to assume that *S. aureus* beta would function with a polymerase, the logical candidate would have been the product of the *dnaE* gene (alpha-small) instead of *polC* (Pol III-L) since the *dnaE* product is more homologous to *E. coli* alpha subunit than Pol III-L.

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Example 10 - The *S. aureus* Beta Subunit Behaves as a Circular Sliding Clamp

The ability of *S. aureus* beta to stimulate Pol III-L could be explained by formation of a 2-protein complex between Pol III-L and beta to form a processive replicase similar to the Type II class (e.g., T7 type). Alternatively, the *S. aureus* replicase is organized as the Type III replicase which operates with a circular sliding clamp and a clamp loader. In this case, the *S. aureus* beta would be a circular protein and would require a clamp loading apparatus to load it onto DNA. The ability of the beta subunit to stimulate Pol III-L in Figure 5A could be explained by the fact that the polydA-oligodT template is a linear DNA and a circular protein could thread itself onto the DNA over an end. Such "end threading" has been observed with PCNA and explains its ability to stimulate DNA polymerase delta in the absence of the RFC clamp loader (Burgers et al., "ATP-Independent Loading of the Proliferating Cell Nuclear Antigen Requires DNA Ends," *J. Biol. Chem.*, 268:19923-19926 (1993), which is hereby incorporated by reference).

20

To distinguish between these possibilities, *S. aureus* beta was examined for ability to stimulate Pol III-L on a circular primed template. In Figure 5B, assays were performed using circular M13mp18 ssDNA coated with *E. coli* SSB and primed with a single oligonucleotide to test the activity of beta on circular DNA. Lane 1 shows the extent of DNA synthesis using Pol III-L alone. In lane 2, Pol III-L was supplemented with *S. aureus* beta. The *S. aureus* beta did not stimulate the activity of Pol III-L on this circular DNA (nor in the absence of SSB). Inability of *S. aureus* beta to stimulate Pol III-L is supported by the results of Figure 6, lane 1 that analyzes the product of Pol III-L action on the circular DNA in an agarose gel in the presence of *S. aureus* beta. In summary, these results show that *S. aureus* beta only stimulates Pol III-L on linear DNA, not circular DNA. Hence, the *S. aureus* beta subunit behaves as a circular protein.

30

Lane 3 shows the result of adding both *S. aureus* beta and *E. coli* gamma complex to Pol III-L. Again, no stimulation was observed (compare with lane 1). This result indicates that the functional contacts between the clamp and clamp loader were not conserved during evolution of Gram positive and Gram negative cells.

5 Controls for these reactions on circular DNA are shown for the *E. coli* system in Lanes 4-6. Addition of only beta to *E. coli* Pol III core did not result in stimulating the polymerase (compare lanes 4 and 5). However, when clamp loader complex was included with beta and core, a large stimulation of synthesis was observed (lane 6). In summary, stimulation of synthesis is only observed when both
10 beta and clamp loader complex were present, consistent with inability of the circular beta ring to assemble onto circular DNA by itself.

Example 11 - Pol III-L Functions as a Pol III-Type Replicase with Beta and a Clamp Loader Complex to Become Processive

15 Next, it was determined whether *S. aureus* Pol III-L requires two components (a beta clamp and a clamp loader) to extend a primer full length around a circular primed template. In Figure 6, a template circular M13mp18 ssDNA primed with a single DNA oligonucleotide was used. DNA products were analyzed in a
20 neutral agarose gel which resolves starting materials (labeled ssDNA in Figure 6) from completed duplex circles (labelled RFII for replicative form II). The first two lanes show, as demonstrated in other examples, that Pol III-L is incapable of extending the primer around the circular DNA in the presence of only *S. aureus* beta. In lane 4 of Figure 6, *E. coli* clamp loader complex (also known as gamma complex)
25 and beta subunit were mixed with *S. aureus* Pol III-L in the assay containing singly primed M13mp18 ssDNA coated with SSB. If the beta clamp, assembled on DNA by clamp loader complex, provides processivity to *S. aureus* Pol III-L, the ssDNA circle should be converted into a fully duplex circle (RFII) which would be visible in an agarose gel analysis. The results of the experiment showed that the *E. coli* beta and
30 clamp loader complex did indeed provide Pol III-L with ability to fully extend the primer around the circular DNA to form the RFII (lane 4). The negative control using only *E. coli* clamp loader complex and beta is shown in lane 3. For comparison, lane 6 shows the result of mixing the three components of the *E. coli* system (Pol III core, beta, and clamp loader complex). This reaction gives almost exclusively full length

RFII product. The qualitatively different product profile that Pol III-L gives in the agarose gel analysis compared to *E. coli* Pol III core with beta and clamp loader complex shows that the products observed using Pol III-L is not due to a contaminant of *E. coli* Pol III core in the *S. aureus* Pol III-L preparation (compare lanes 4 and 6).

5 It is generally thought that the polymerase of one system is specific for its SSB. However, these reactions are performed on ssDNA coated with the *E. coli* SSB protein. Hence, the *S. aureus* Pol III-L appears capable of utilizing *E. coli* SSB and the *E. coli* beta. It would appear that the only component that is not interchangeable between the Gram positive and Gram negative systems is the clamp loader complex.

10 Thus, the *S. aureus* Pol III-L functions as a Pol III type replicase with the *E. coli* beta clamp assembled onto DNA by a clamp loader complex.

15 **Example 12 - Purification of Two DNA Polymerase III-Type Enzymes From *S. aureus* Cells**

The MonoQ resin by Pharmacia has very high resolution which would resolve the three DNA polymerases of *S. aureus*. Hence, *S. aureus* cells were lysed, DNA was removed from the lysate, and the clarified lysate was applied onto a MonoQ column. The details of this procedure are: 300 L of *S. aureus* (strain 4220, a gift of Dr. Pat Schlievert, University of Minnesota) was grown in 2X LB media at 37°C to an O.D. of approximately 1.5 and then were collected by centrifugation. Approximately 2 kg of wet cell paste was obtained and stored at -70°C. 122 g of cell paste was thawed and resuspended in 192 ml of cell lysis buffer followed by passage through a French Press cell (Aminco) at 40,000 psi. The resultant lysate was clarified by high speed centrifugation (1.3 g protein in 120 ml). A 20 ml aliquot of the supernatant was dialyzed 2 h against 2 L of buffer A containing 50 mM NaCl. The dialyzed material (148 mg, conductivity = 101 mM NaCl) was diluted 2-fold with Buffer A containing 50 mM NaCl and then loaded onto an 8 ml MonoQ column equilibrated in Buffer A containing 50 mM NaCl. The column was washed with Buffer A containing 50 mM NaCl, and then eluted with a 160 ml linear gradient of 0.05 M NaCl to 0.5 M NaCl in Buffer A. Fractions of 2.5 ml (64 total) were collected, followed by analysis in an SDS polyacrylamide gel for their replication activity in assays using calf thymus DNA.

Three peaks of DNA polymerase activity were identified (Figure 7). Previous studies of cell extracts prepared from the Gram positive organism *Bacillus subtilis* identified only two peaks of activity off a DEAE column (similar charged resin to MonoQ). The first peak was Pol II, and the second peak was a combination of DNA polymerases I and III. The DNA polymerases I and III were then separated on a subsequent phosphocellulose column. The middle peak in Figure 7 is much larger than the other two peaks and, thus, it was decided to chromatograph this peak on a phosphocellulose column. The second peak of DNA synthetic activity was pooled (fractions 37-43; 28 mg in 14 ml) and dialyzed against 1.5 L P-cell buffer for 2.5 h. Then, the sample (ionic strength equal to 99 mM NaCl) was applied to a 5 ml phosphocellulose column equilibrated in P-cell buffer. After washing the column in 10 ml P-cell buffer, the column was eluted with a 60 ml gradient of 0 - 0.5 M NaCl in P-cell buffer. Seventy fractions were collected and then analyzed for DNA synthesis using calf thymus DNA as template. This column resolved the polymerase activity into two distinct peaks (Figure 7B).

Hence, there appear to be four DNA polymerases in *Staphylococcus aureus*. They were designated here as peak 1 (first peak off MonoQ), peak 2 (first peak off phosphocellulose), peak 3 (second peak of phosphocellulose), and peak 4 (last peak off Mono Q) (see Figure 7). Peak 4 was presumably Pol III-L, as it elutes from MonoQ in a similar position as the Pol III-L expressed in *E. coli* (compare Figure 7A with Figure 2).

Example 13 - Demonstration That Peak 1 (Pol III-2) Functions as a Pol III-Type Replicase With *E. coli* Beta Assembled on DNA by *E. coli* Clamp Loader Complex.

To test which peak contained a Pol III-type of polymerase, an assay was used in which the *E. coli* clamp loader complex and beta support formation of full length RFII product starting from *E. coli* SSB coated circular M13mp18 ssDNA primed with a single oligonucleotide. In Figure 8, both Peaks 1 and 2 are stimulated by the *E. coli* clamp loader complex and beta subunit and, in fact, Peaks 2 and 3 are inhibited by these proteins (the quantitation is shown below the gel in the figure). Further, the product analysis in the agarose gel shows full length RFII duplex DNA circles only for peaks 1 and 4. These results, combined with the NEM, pCMB, and

KCl characteristics in Tables 2 and 3 below, suggest that there are two Pol III-type DNA polymerases in *S. aureus* and that these are partially purified in peaks 1 and 4.

Next, it was determined which of these peaks of DNA polymerase activity correspond to DNA polymerases I, II, and III, and which peak is the unidentified DNA polymerase. In the Gram positive bacterium *B. subtilis*, Pol III is inhibited by pCMB, NEM, and 0.15 M NaCl, Pol II is inhibited by KCl, but not NEM or 0.15 M KCL, and Pol I is not inhibited by any of these treatments (Gass et al., "Further Genetic and Enzymological Characterization of the Three *Bacillus subtilis* Deoxyribonucleic Acid Polymerases," *J. Biol. Chem.*, 248:7688-7700 (1973), which is hereby incorporated by reference). Hence, assays were performed in the presence or absence of pCMB, NEM, and 0.15 M KCl (see Tables 2 and 3 below). Peak 3 clearly corresponded to Pol I, because it was not inhibited by NEM, pCMB, or 0.15 M NaCl. Peak 2 correspond to Pol II, because it was not inhibited by NEM, but was inhibited by pCMB and 0.15 M NaCl. Peaks 1 and 4 both had characteristics that mimic Pol III; however, peak 4 elutes on MonoQ at a similar position as Pol III-L expressed in *E. coli* (see Figure 2B). Hence, peak 4 is likely Pol III-L, and peak 1 is likely the unknown polymerase.

Table 2: Expected Characteristics of Polymerases

Polymerase	pCMB	NEM	0.15M KCl
Pol I	not inhibited*	not inhibited	not inhibited
Pol II	inhibited**	not inhibited	not inhibited
Pol III-L	inhibited	inhibited	not inhibited

* Not inhibited is defined as greater than 75% remaining activity

** Inhibited is defined as less than 40% remaining activity

Table 3: Observed Characteristics

Peak	pCMB	NEM	0.15M KCL assignment
Peak1	inhibited	inhibited	new polymerase
Peak2	inhibited	not inhibited	Pol II
Peak3	not inhibited	not inhibited	Pol I
Peak4	inhibited	inhibited	Pol III-L

Example 14 - Identification and Cloning of *S. aureus* dnaE

This invention describes the finding of two DNA polymerases that function with a sliding clamp assembled onto DNA by a clamp loader. One of these DNA polymerases is likely Pol III-L, but the other has not been identified previously. Presumably, the chromatographic resins used in earlier studies did not have the resolving power to separate the enzyme from other polymerases. This would be compounded by the low activity of Pol III-2. To identify a gene encoding the second Pol III, the amino acid sequences of the Pol III alpha subunit of *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Haemophilis influenzae*, and *Helicobacter pylori* were aligned using Clustal W (1.5). Two regions about 400 residues apart were conserved and primers were designed for the following amino acid sequences:

Upstream, corresponding in *E. coli* to residues 385-399 (SEQ. ID. No. 43)

Leu Leu Phe Glu Arg Phe Leu Asn Pro Glu Arg Val Ser Met Pro
1 5 10 15

Downstream, corresponding in *E. coli* to residues 750-764 (SEQ. ID. No. 44)

Lys Phe Ala Gly Tyr Gly Phe Asn Lys Ser His Ser Ala Ala Tyr
1 5 10 15

The following primers were designed to these two peptide regions using codon preferences for *S. aureus*:

Upstream (SEQ. ID. No. 45)

cttcttttttg aaagatttct aaataaagaa cgttattcaa tgcc 44

Downstream (SEQ. ID. No. 46)

ataagctgca gcatgacttt tattaaaacc ataacctgca aattt 45

Amplification was performed using 2.5 units of *Taq* DNA Polymerase (Gibco, BRL), 100 ng *S. aureus* genomic DNA, 1 mM of each of the four dNTPs, 1 μ M of each primer, and 3 mM MgCl₂ in 100 μ l of *Taq* buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 90 sec. The PCR product (approximately 1.1 kb) was electrophoresed in a 0.8 % agarose gel and purified using

a GeneClean III kit (Bio 101). The product was then divided equally into ten separate aliquots and used as a template for PCR reactions, according to the above protocol, to reamplify the fragment for sequencing. The final PCR product was purified using a Quiagen Quiaquick PCR Purification kit, quantitated via optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reactions.

Next, the following additional PCR primers were designed to obtain more sequence information 3' to the first amplified section.

Upstream (SEQ. ID. No. 47)

agttaaaaat gccatatttt gacgtgtttt agttctaataat

39

Downstream (SEQ. ID. No. 48)

cttgcaaaaag cggttgctaa agatggttga cgaattatgg gg

42

These primers were used in a PCR reaction using 2.5 units of *Taq* DNA Polymerase (Gibco, BRL) with 100 ng *S. aureus* genomic DNA as a template, 1mM dNTP's, 1 μ M of each primer, and 3 mM MgCl₂ in 100 l of *Taq* buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min; 55°C, 1 min; 72°C, 2 min 30 seconds. The 1.6 Kb product was then divided into 5 aliquots, and used as a template in a set of 5 PCR reactions, as described above, to amplify the product for sequencing. The products of these reactions were purified using a Qiagen Quiaquick PCR Purification kit, quantitated via optical density at 260 nm, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The sequence of this product yielded about 740 bp of new sequence 3' of the first sequence.

As this gene shows better homology to the Gram negative Pol III α subunit compared to Gram positive Pol III-L, it will be designated the *dnaE* gene.

Example 15 - Identification and Cloning of *S. aureus dnaX*

The fact that the *S. aureus* beta stimulates Pol III-L and has a ring shape suggests that the Gram postive replication machinery is of the three component type. This implies the presence of a clamp loader complex. This is not a simple

determination to make as the *B. subtilis* genome shows homologs to only two of the five subunits of the *E. coli* clamp loader (*dnaX* encoding gamma, and *holB* encoding delta prime). On the basis of the experiments in this application, which suggests that there is a clamp loader, it was believed that these two subunit homologues are part of the clamp loader for the *S. aureus* beta.

As a start in obtaining the clamp loading apparatus, a strategy was devised to obtain the gene encoding the tau subunit of *S. aureus*. In *E. coli*, the tau and gamma subunits are derived from the same gene. Tau is the full length product, and gamma is about 2/3 the length of tau. Gamma is derived from the *dnaX* gene by what was originally believed to be an efficient translational frameshift mechanism that, after it occurs, incorporates only one unique C-terminal residue before encountering a stop codon. To identify the *dnaX* gene of *S. aureus* by PCR analysis, the *dnaX* genes of *B. subtilis*, *E. coli*, and *H. influenzae* were aligned. Upon comparison of the amino acid sequence encoded by these *dnaX* genes, two areas of high homology were used to predict the amino acid sequence of the *S. aureus dnaX* gene product. PCR primers were designed to these sequences, and a PCR product of the expected size was indeed produced. DNA primers were designed to two regions of high similarity for use in PCR that were about 100 residues apart. The amino acid sequences of these regions were:

Upstream, corresponding to residues 39-48 of *E. coli* (SEQ. ID. No. 49)

His	Ala	Tyr	Leu	Phe	Ser	Gly	Pro	Arg	Gly
1					5				10

Downstream, corresponding to residues 138-148 of *E. coli* (SEQ. ID. No. 50)

His	Ala	Tyr	Leu	Phe	Ser	Gly	Pro	Arg	Gly
1					5				10

The DNA sequence of the PCR primers was based upon the codon usage of *S. aureus*.

The primers are as follows:

Upstream (SEQ. ID. No. 51)

cgcggatccc atgcatatattt attttcaggt ccaagagg

Downstream (SEQ. ID. No. 52)

ccggaattct ggtggttctt ctaatgtttt taataatgc

39

5 The first 9 nucleotides of the upstream primer (SEQ. ID. No. 51) contain a BamHI site, which is underlined, and do not correspond to amino acid codons; the 3' 29 nucleotides correspond to the amino acid sequence of SEQ. ID. No. 49. The EcoRI site of the downstream primer (SEQ. ID. No. 52) is underlined and the 3' 33 nucleotides correspond to the amino acid sequence of SEQ. ID. No. 50.

10 The expected PCR product, based on the alignment, is approximately 268 bp between the primer sequences. Amplification was performed using 500 ng genomic DNA, 0.5 mM dNTPs, 1 μ M of each primer, 1 mM MgSO₄, 2 units vent DNA polymerase in 100 μ l of vent buffer. Forty cycles were performed using the following cycling scheme: 94°C, 1 min; 60°C, 1 min.; 72°C, 30s. The approximately 300 bp product was digested with EcoRI and BamHI and purified in a 0.7 % agarose gel. The pure digested fragment was ligated into pUC18 which had been digested with EcoRI and BamHI and gel purified in a 0.7 % agarose gel. Ligated products were transformed into *E. coli* competent DH5 α cells (Stratagene), and colonies were screened for the correct chimera by examining minipreps for proper length and correct digestion products using EcoRI and BamHI. The sequence of the insert was

15 determined and was found to have high homology to the *dnaX* genes of several bacteria. This sequence was used to design circular PCR primers. Two new primers were designed for circular PCR based on this sequence.

20

25 A circular PCR product of approximately 1.6 kb was obtained from a HincII digest of chromosomal DNA that was recircularized with ligase. This first circular PCR yielded most of the remaining *dnaX* gene. The two primers were as follows:

Rightward (SEQ. ID. No. 53)

tttgtaaagg cattacgcag gggactaatt cagatgtg

38

30

Leftward (SEQ. ID. No. 54)

tatgacattc attacaaggt tctccatcag tgc

33

Genomic DNA (3 µg) was digested with HincII, purified with phenol/chloroform extraction, ethanol precipitated and redissolved in 70 µl T.E. buffer. The genomic DNA was recircularized upon adding 4000 units T4 ligase (New England Biolabs) in a final volume of 100 µl T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 µl elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min.; 55°C, 1 min.; and 68°C, 2 min. The resulting PCR product was approximately 1.6 kb. The PCR product was purified from a 0.7 % agarose gel and sequenced directly. A stretch of approximately 750 nucleotides was obtained using the rightward primer used in the circular PCR reaction. To obtain the rest of the sequence, other sequencing primers were designed in succession based on the information of each new sequencing run.

This sequence, when spliced together with the previous 300 bp PCR sequence, contained the complete N-terminus of the gene product (stop codons are present upstream) and possibly lacked only about 50 residues of the C-terminus. The amino terminal region of *E. coli* tau shares what appears to be the most conserved region of the gene as this area shares homology with RFC subunit of the human clamp loader and with the gene 44 protein of the phage T4 clamp loader. An alignment of the N-terminal region of the *S. aureus* tau protein with that of *B. subtilis* and *E. coli* is shown in Figure 10. Among the highly conserved residues are the ATP binding site consensus sequence and the four cystine residues that form a Zn²⁺ finger.

After obtaining 1 kb of sequence in the 5' region of *dnaX*, it was sought to determine the remaining 3' end of the gene. Circular PCR products of approximately 800bps, 600bps, and 1600bps were obtained from Apo I, or Nsi I or Ssp I digest of chromosomal DNA that were recircularized with ligase.

Rightward (SEQ. ID. No. 55)

gagcactgat gaacttagaa ttagatatg

29

Leftward (SEQ. ID. No. 56)

gatactcagt atctttctca gatgttttat tc

32

Genomic DNA (3 g) was digested with, Apo I, or Nsi I or Ssp I, purified with phenol/chloroform extraction, ethanol precipitated, and redissolved in 70 l T.E. buffer. The genomic DNA was recircularized upon adding 4000 units of T4 ligase (New England Biolabs) in a final volume of 100 l T4 ligase buffer (New England Biolabs) at 16°C overnight. The PCR reaction consisted of 90 ng recircularized genomic DNA, 0.5 mM each dNTP, 100 pmol of each primer, 1.4 mM magnesium sulfate, and 1 unit of elongase (GIBCO) in a final volume of 100 l elongase buffer (GIBCO). 40 cycles were performed using the following scheme: 94°C, 1 min.; 55°C, 1 min.; 68°C, 2 min. The PCR products were directly cloned into pCR II TOPO vector using the TOPO TA cloning kit (Invitrogen Corporation) for obtaining the rest of the C terminal sequence of *S. aureus dnaX*. DNA sequencing was performed by the Rockefeller University sequencing facility.

Example 16 - Identification and Cloning of *S. aureus dnaB*

In *E. coli*, the DnaB helicase assembles with the DNA polymerase III holoenzyme to form a replisome assembly. The DnaB helicase also interacts directly with the primase to complete the machinery needed to duplicate a double helix. As a first step in studying how the *S. aureus* helicase acts with the replicase and primase, *S. aureus* was examined for presence of a *dnaB* gene.

The amino acid sequences of the DnaB helicase of *Escherichia coli*, *Salmonella typhimurium*, *Haemophilis influenzae*, and *Helicobacter pylori* were aligned using Clustal W (1.5). Two regions about 200 residues apart showed good homology. These peptide sequences were:

Upstream, corresponding to residues 225-238 of *E. coli* DnaB (SEQ. ID. No. 57)

Asp Leu Ile Ile Val Ala Ala Arg Pro Ser Met Gly Lys Thr
1 5 10

Downstream, corresponding to residues 435-449 of *E. coli* DnaB (SEQ. ID. No. 58)

Glu Ile Ile Ile Gly Lys Gln Arg Asn Gly Pro Ile Gly Thr Val
1 5 10 15

The following primers were designed from regions which contained conserved sequences using codon preferences for *S. aureus*:

Upstream (SEQ. ID. No. 59)

gaccttataa ttgtagctgc acgtccttct atgggaaaaa c 41

5 Downstream (SEQ. ID. No. 60)

aacattatta agtcagcatc ttgttctatt gatccagatt caacgaag 48

A PCR reaction was carried out using 2.5 units of *Taq* DNA Polymerase (Gibco, BRL) with 100 ng. *S. aureus* genomic DNA as template, 1 mM dNTP's, 1 μ M of each primer, 3 mM MgCl₂ in 100 μ l of *Taq* buffer. Thirty-five cycles of the following scheme were repeated: 94°C, 1 min.; 55°C, 1 min.; and 72°C, 1 min. Two PCR products were produced, one was about 1.1 kb, and another was 0.6 kb. The smaller one was the size expected. The 0.6 kb product was gel purified and used as a template for a second round of PCR as follows. The 0.6 kb PCR product was purified from a 0.8% agarose gel using a GeneClean III kit (Bio 101) and then divided equally into five separate aliquots, as a template for PCR reactions. The final PCR product was purified using a Quiagen Quiaquick PCR Purification kit, quantitated via optical density at 260 nM, and sequenced by the Protein/DNA Technology Center at Rockefeller University. The same primers used for PCR were used to prime the sequencing reaction. The amino acid sequence was determined by translation of the DNA sequence in all three reading frames, and selecting the longest open reading frame. The PCR product contained an open reading frame over its entire length. The predicted amino acid sequence shares homology to the amino acid sequences encoded by *dnaB* gene of other organisms.

Additional sequence information was determined using the circular PCR technique. Briefly, *S. aureus* genomic DNA was digested with various endonucleases, then religated with T4 DNA ligase to form circular templates. To perform PCR, two primers were designed from the initial sequence.

30 First primer (SEQ. ID. No. 61)

gattttagt tctggtaatg ttgactcaaa ccgcttaaga accgg 45

Second primer (SEQ. ID. No. 62)

atacgtgtgg ttaactgatc agcaacccat ctctagttag aaaatacc 48

The first primer matches the sequence of the coding strand and the second primer matches the sequence of the complementary strand. These two primers are directed outwards from a central point, and allow determination of new sequence information up to the ligated endonuclease site. A PCR product of approximately 900 bases in length was produced using the above primers and template derived from the ligation of *S. aureus* genomic DNA which had been cut with the restriction endonuclease Apo I. This PCR product was electrophoresed in a 0.8% agarose gel, eluted with a Qiagen gel elution kit, divided into five separate aliquots, and used as a template for reamplification by PCR using the same primers as described above. The final product was electrophoresed in an 0.8% agarose gel, visualized via staining with ethidium bromide under ultraviolet light, and excised from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm for 15 minutes. The supernatant was extracted with phenol/chloroform to remove ethidium bromide, and was then cleaned using a Qiagen PCR purification kit. The material was then quantitated from its optical density at 260 nm and sequenced by the Protein/DNA Technology Center at the Rockefeller University.

The nucleotide sequence contained an open reading frame over its length, up to a sequence which corresponded to the consensus sequence of a cleavage site of the enzyme Apo I. Following this point, a second open reading frame encoded a different reading frame up to the end of the product. The initial sequence information was found to match the initial sequence and to extend it yet further towards the C-terminus of the protein. The second reading frame was found to end in a sequence which matched the 5'-terminus of the previously determined sequence and, thus, represents an extension of the sequence towards the N-terminus of the protein.

Additional sequence information was obtained using the above primers and a template generated using *S. aureus* genomic DNA circularized via ligation with T4 ligase following digestion with Cla I. The PCR product was generated using 35 cycles of the following program: denaturation at 94°C for 1 min.; annealing at 55°C for 1 min.; and extension at 68°C for 3 minutes and 30 s. The PCR products were electrophoresed in a 0.8% agarose gel, eluted with a Qiagen gel elution kit, divided into five separate aliquots, and used as a template reamplification via PCR with the same primers described above. The final product was electrophoresed in an 0.8%

agarose gel, visualized via staining with ethidium bromide under ultraviolet light, and excised from the gel. The excised gel slice was frozen, and centrifuged at 12,000 rpm for 15 min. The supernatant was cleaned using a Qiagen PCR purification kit. The material was then quantitated via optical density at 260 nm and sequenced by the Protein/DNA Technology Center at Rockefeller University. The open reading frames continued past 500 bases. Therefore, the following additional sequencing primers were designed from the sequence to obtain further information:

First primer (SEQ. ID. No. 63)

10 cgttttaatg catgcttaga aacgatatca g 31

Second primer (SEQ. ID. No. 64)

cattgctaag caacgttacg gtccaacagg c 31

15 The N-terminal and C-terminal nucleotide sequence extensions generated using this circular PCR product completed the 5' region of the gene (encoding the N-terminus of DnaB); however, a stop codon was not reached in the 3' region and, thus, a small amount of sequence is still needed to complete this gene.

20 The alignment of the *S. aureus dnaB* with *E. coli dnaB* and the *dnaB* genes of *B. subtilis* and *S. typhimurium* is shown in Figure 11.

Example 17 - Identification and Cloning of *S. aureus holB*

25 The *S. aureus holB* was identified by searching the *S. aureus* database with the sequences of *S. pyogenes* δ' subunit. The *S. aureus holB* encodes a 253 residue protein of about 28 kDa. The *holB* gene was amplified by PCR using an upstream 69-mer primer as follows:

Upstream Primer (SEQ. ID. No. 65):

30 ggataacaat tccccgctag caataatttt gttaaacttt aagaaggaga tatacccatg 60
gatgaacag 69

which contains an *NcoI* site (underlined), and a downstream 39-mer primer as follows:

Downstream Primer (SEQ. ID. No. 66):

aattttaaag gatccgtgta taatattcta attttcccg

39

5 which contains a *Bam*HI site (underlined). The PCR product was digested with *Nco*I and *Bam*HI, purified, and ligated into the *Nco*I and *Bam*HI sites of pET11a to produce plasmid pETSaholB.

Example 18 - Purification of *S. aureus* δ'

10

The pETSaholB plasmid of Example 17 was transformed into *E. coli* BL21(DE3)*recA*. A single colony was used to inoculate 2L of LB media supplemented with 200 μ g/ml ampicillin. Cells (2L) were grown at 37°C to OD₆₀₀=0.5 at which point the temperature was lowered to 15°C and 0.5 mM IPTG was added. After 16 hr of induction, cells were collected by centrifugation, resuspended in 50 mM Tris-HCl (pH 7.5), 10% sucrose, 1 M NaCl, 30 mM spermidine, 5 mM DTT, and 2 mM EDTA. Cells were lysed by two passages through a French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min at 4°C. Ammonium sulfate (0.3 g/ml) was added to the clarified lysate. The pellet was backwashed in 30 ml buffer A containing 0.1 M NaCl and 0.24 g/ml ammonium sulfate using a Dounce homogenizer, then the pellet was recovered by centrifugation. The resulting pellet was resuspended in 20 ml of buffer A and dialyzed against buffer A. The dialyzed protein was applied to a 20 ml FFQ Sepharose column equilibrated in buffer A and eluted with a 200 ml linear gradient of 0 - 500 mM NaCl in buffer A; 80 fractions were collected. Peak fractions (54 - 75) were combined (72 mg) and dialyzed against buffer A. The δ' preparation was aliquoted and stored frozen at -80°C.

15

20

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Example 19 - Identification and Cloning of *S. aureus* *hola*

30

The *S. aureus* *hola* gene was identified by searching the *S. aureus* database with the sequences of *E. coli* and *S. pyogenes* δ subunits. The *S. aureus* *hola*

The pETSaholaA plasmid of Example 19 was transformed into *E. coli* NovaBlue (*recA1 lac[F'proA⁺B⁺ lac^gZAM15::Tn10(Tc^R)*) (Novagen). A single colony was used to inoculate 12L of LB media supplemented with 200 µg/ml ampicillin. Cells (12L) were grown at 37°C to OD₆₀₀=0.5 at which point the temperature was lowered to 15°C and 0.5 mM IPTG was added. After 16 hr of induction, cells were collected by centrifugation, resuspended in 50 mM Tris-HCl (pH 7.5), 10% sucrose, 1M NaCl, 30 mM spermidine, 5 mM DTT, and 2 mM EDTA. Cells were lysed by two passages through a French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min at 4°C. Ammonium sulfate (0.3 g/ml) was added to the clarified lysate. The resulting pellet was resuspended in 250 ml of buffer A. The dialyzed protein was applied to a 100 ml FFQ Sepharose column equilibrated in buffer A and eluted with a 1000 ml linear gradient of 0 - 500 mM NaCl in buffer A; 80 fractions were collected. Peak fractions (40-49) were combined (65 mg) and dialyzed against buffer A. The dialyzed protein was applied to a 8 ml MonoQ Sepharose column equilibrated in buffer A and eluted with a 80 ml linear gradient of 0

- 500 mM NaCl in buffer A; 80 fractions were collected. Peak fractions of the δ preparation were stored frozen at -80°C .

5 **Example 21 - Consitution of a Processive *S. aureus* DNA Polymerase III Enzyme from Three Components**

10 The PolC (alpha-large) requires the β clamp for processivity, which in turn requires the clamp loader ($\tau\delta\delta'$) for assembly onto DNA. The *S. aureus* clamp loader, $\tau\delta\delta'$ complex, was assembled by mixing the three proteins as follows: 400 μg of τ and 80 μg each of δ and δ' were mixed in buffer A containing no NaCl and preincubated at 15°C for 10 min. The mixture was injected onto a 1 ml MonoQ column equilibrated in buffer A, and then eluted with a 30 ml linear gradient of 0-500 mM NaCl in buffer A; 60 fractions were collected. Fractions were analyzed in a 10% SDS-polyacrylamide gel stained with Coomassie Blue. Peak fractions (40-50) were
15 combined and concentrated using a Centricon 30 concentrator.

20 The ability of the three components to work together to form the processive Pol III was tested by determining whether $\tau\delta\delta'$ and β clamp could confer the ability of PolC to completely extend a single primer full circle around a large 7.2 kb circular M13mp18 ssDNA genome. Replication reaction contained 70 ng (25 fmol) on singly primed M13mp18 ssDNA, 20 ng *S. aureus* β , 50 ng *S. aureus* PolC, either 30 ng or 90 ng of *S. aureus* $\tau\delta\delta'$ (when indicated), and 0.82 μg of *S. pyogenes* SSB in 24 μl of 20 mM Tris-HCl (pH 7.5), 4% glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM MgCl_2 , 40 $\mu\text{g}/\text{ml}$ BSA, and 60 mM each of dGTP and dCTP. Reactions were pre-incubated for 2 min at 37°C to assemble protein complexes on the
25 primer terminus. DNA synthesis was initiated upon addition of 1.5 μl dATP and ^{32}P -TTP (specific activity 2,000-4,000 cpm/pmol) and synthesis was allowed to proceed for 1 min before being quenched with an equal volume (25 μl) of a solution of 1% SDS and 40 mM EDTA. One-half of the quenched reaction was analyzed for total DNA synthesis using DE81 paper as described, and the other half was analyzed by
30 agarose gel phoresis. An autoradiogram of the agarose gel analysis of the replication products is depicted in Figure 13, which shows that the presence of PolC and β , but absence of $\tau\delta\delta'$ (lane 1) gives no full length circular duplex (RFII). However, in the

presence of $\tau\delta\delta'$ (lanes 2 and 3), full length circular duplex DNA (RFII) is produced, as expected for the action of a processive Pol III holozyyme.

Example 22 - General Induction/Purification Conditions for *S. pyogenes*

5 The purification protocols for *S. pyogenes* proteins were performed using following standardized conditions. Cells were grown from a single colony, freshly transformed overnight. Cells were grown in 200 μ g/ml Ampicillin to OD600=0.3-0.4, at which point cultures were chilled prior to addition of IPTG (to a
10 final concentration of 0.5 mM) and were allowed to incubate for 16 hrs at 15°C. Following this, all procedures were performed at 4°C. Cell paste (1-2 g/liter of culture) was resuspended (10 ml/g cell paste) in 50 mM Tris-HCl (pH 7.5)/10% Sucrose/1 M NaCl/5 mM DTT/ 30 mM Spermidine/1X Heat lysis buffer (50 mM Tris-HCl (pH 7.5), 1% Sucrose, 100 mM NaCl, 2 mM EDTA). Cells were lysed by
15 two passages through the French Press (15,000 psi) followed by centrifugation at 14,000 rpm at 4 °C. Ammonium sulfate, when added to the cleared lysate, was added gradually. Precipitate was allowed to settle on ice for a minimum of 30 min prior to collection by centrifugation. Protein pellets were resuspended in buffer A (50 mM Tris-HCl pH 7.5, 1 mM EDTA, 5 mM DTT, 10% glycerol) and dialyzed for over 3
20 hours in the same buffer. Column design is based on the manufacturer's suggested capacities: Fast Flow Q (FFQ) and MonoQ are 20 mg protein /ml resin, Heparin-Affigel agarose is 1.2 mg protein/ml resin. Elution was performed using 10 column volume (c.v.) gradients, and the entire gradient elution profile was collected in 80 fractions. Unless mentioned otherwise all columns were equilibrated and eluted with
25 buffer A.

Example 23 - Identification of a *S. pyogenes* *holA* gene Encoding a Functional Delta Subunit and Purification of the Delta Subunit

30 Alignment of *E. coli* delta subunit with 10 other putative *holA* products from unfinished genome databases of Gram negative bacteria indicates a region of conserved amino acid sequence. Amino acids Q140 to L230 of *E. coli* delta were used to search the *B. subtilis* genome database for a Gram positive delta homolog. This search revealed *yqeN*, a potential reading frame of unknown function, as the

highest scoring sequence. Although the score was low, it was treated as a candidate for Gram positive delta. The alignment with *E. coli* delta is shown in Figure 12A. A *Streptococcus pyogenes* genome database was searched with *yqeN*. Two contigs which represent N- (contig 206) and C- (contig 264) termini of *S. pyogenes* delta subunit were identified. The alignment of the putative *S. pyogenes* *holA* with *B. subtilis* *yqeN* is shown in Figure 12B. The following primers were used to obtain PCR products for delta subunit:

holA Upstream (SEQ. ID No. 69)

10 ggagcagatt gcttttgata catatgattg gcctattc 38

holA Downstream (SEQ. ID No. 70)

ttgtctccgc atcaaactgg gatccaagag catcatacgc gtatgg 46

15 These primers were used to amplify the *holA* gene from *S. pyogenes* genomic DNA. The PCR product was digested with NdeI and BamHI, purified and ligated into the pET11a vector to produce pET11a.S.p. *holA*.

The pET11a.S.p.*holA* plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 12L LB broth supplemented with 200 µg/ml Ampicillin. Cells were grown at 37°C to OD600=0.5, at which point the temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning, cells were collected by centrifugation and resuspended in 50 mM Tris-HCl (pH 7.5)/10% Sucrose /1X Heat Lysis Buffer/1M NaCl/30 mM Spermidine/5 mM DTT. Cells were lysed by two passages through the French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min. The supernatant was decanted and ammonium sulfate was added to a final concentration of 0.226 g/ml. The resulting pellet was collected by centrifugation and resuspended in 20 ml of buffer A. The resuspended pellet was dialyzed against buffer A containing no salt. The dialyzed protein (500 mg) was loaded onto a FFQ- Sepharose (35 ml) column and eluted with a linear gradient from 0 - 500 mM NaCl (10 c.v.). The peak fractions (21-45) were combined and dialyzed against buffer A (0 NaCl) for 3 hrs, then diluted to a conductivity of 50 mM NaCl and loaded (160 mg) onto a 120 ml Heparin-Affigel

column. Protein was eluted with a linear gradient of 0-500 mM NaCl (10 c.v.). The fractions containing the least contaminants (39-51) were precipitated with ammonium sulfate (0.226 g), collected by centrifugation, resuspended 5 ml of buffer A, and dialyzed in buffer A containing 200 mM NaCl. The delta subunit was stored at -80°C. The final delta subunit preparation is shown in the lane marked δ of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 65 mg.

Example 24 - Identification of *S. pyogenes* *holB* Encoding Delta Prime and Purification of the Delta Prime Subunit

A search of the *S. pyogenes* genome database with the predicted *B. subtilis* delta prime amino acid sequence revealed a DNA sequence in contig #209 (previously known as contig # 210) that predicted a high scoring match for a gene encoding a delta prime protein. The following primers were used to obtain PCR products for *holB*:

***holB* Upstream** (SEQ. ID. No. 71)

gcctaggata agggagggtg catatggatt tagcgc 36

***holB* Downstream** (SEQ. ID. No. 72)

cgggcaagtc ttttgacaag cttcggatcc ccataacgaa ttcc 44

The PCR product obtained from these primers was digested with NdeI and BamHI, purified and ligated into the pET11a vector to produce pET11a.S.p. *holB*.

The pET11a.S.p.*holB* plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 12L LB broth supplemented with 200 μ g/ml Ampicillin. Cells were grown at 37°C to O.D.600=0.4, at which point the temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning, cells were collected by centrifugation and resuspended in 100 ml 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer. Lysis was initiated upon addition of 0.4 mg/ml lysozyme followed by a 1 hr incubation on ice. Lysate was clarified by centrifugation at 13,000 rpm for 30 min. Ammonium sulfate was added to the supernatant to a final concentration of 0.3 g/ml. The protein pellet was resuspended in

buffer A(0.1 M NaCl) + 0.24 g/ml ammonium sulfate and clarified by centrifugation. The resulting protein pellet was resuspended in 20 ml of buffer A and dialyzed against buffer A. The dialyzed protein (450 mg) was loaded onto a 30 ml FFQ- Sepharose column and eluted with a linear gradient from 0 - 500 mM NaCl. The peak fractions were combined (fr# 20-30 containing 130 mg) and dialyzed against buffer A and loaded (70 mg) onto a 50 ml Heparin-Affigel column. Protein was eluted with a linear gradient of 0-500 mM NaCl. Delta prime binds weakly to both resins and elutes in the beginning of the gradient. This delta prime subunit was stored frozen at - 80°C. The final delta prime subunit preparation is shown in lane marked δ' of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 40 mg.

Example 25 - Identification of the *S. pyogenes dnaX* Gene and Purification of the Tau Subunit

A search of the *S. pyogenes* genome database with the putative *B. subtilis* tau amino acid sequence revealed a DNA sequence in contig #284 (previously known as contig # 289) with a high scoring match which predicted a gene encoding for a tau subunit protein. A set of PCR primers to 5'- and 3'- termini of the putative gene sequence were designed to include restriction enzyme recognition sequences for NdeI and BamHI sites, respectively. These primers are:

dnaX Upstream (SEQ. ID. No. 73)

ggaggttaaaa acatatgtat caagctcttt atc 33

dnaX Downstream (SEQ. ID. No. 74)

cgtgggtaag ggcaaaacgg atcccttatg tatttcag 38

A PCR product obtained with the above primers was digested with NdeI and BamHI, purified and ligated into pET11a vector to produce pET11a.S.p.dnaX.

The pET11a.S.p.dnaX plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 24L LB broth supplemented with 200 μ g/ml Ampicillin. Cells were grown at 37°C to O.D.600=0.5, at which point the temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning,

cells were collected by centrifugation and resuspended in 200 mls of 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer/1M NaCl/30 mM Spermidine/5 mM DTT/5 mM EDTA. Cells were lysed by two passages through the French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min. The supernatant (2.4 gm) was dialyzed against buffer A containing 50 mM NaCl, loaded onto a 120 ml FFQ column (without ammonium sulfate precipitation) and eluted with a linear gradient of 100-700 mM NaCl. The peak fractions (fr# 41-55) were combined, diluted with buffer A containing no salt (a dilution of 1/5) to a conductivity of 100 mM NaCl, loaded (310 mg) onto a 300 ml Heparin-Affigel column, and eluted with a linear gradient of 100-500 mM NaCl. The peak fractions (fr# 21-36) were combined, dialyzed against buffer A, loaded (87 mg) onto 10 ml FFQ column, and eluted as described for the first FFQ column. The peak fractions (fr# 27-41) were concentrated by centrifugation in Centriprep 30 filtration unit and frozen at -80°C. The final tau subunit preparation is shown in the lane marked τ of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 103 mg.

Example 26 - Identification of the *S. pyogenes* *dnaN* Gene and Purification of the Beta Subunit

A search of the *S. pyogenes* genome database with the putative *B. subtilis* beta subunit amino acid sequence revealed a DNA sequence (contig # 266) with a high scoring match which predicted a gene encoding for a beta subunit protein. A set of PCR primers to 5'- and 3'- termini of the putative gene sequence were designed to include restriction enzyme recognition sequences for NdeI and BamHI, respectively. The primers were:

***dnaN* Upstream** (SEQ. ID. No. 75)

ggagttcata tgattcaatt ttcaaattaa tcgc

34

***dnaN* Downstream** (SEQ. ID. No. 76)

tatcagctcc tggatccagt accttcatt gattagcc

38

A PCR product obtained with these primers was digested with NdeI and BamHI, purified and ligated into pET16b vector to produce pET16b.S.p.dnaN.

The pET16b.S.p.dnaN plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 15L LB broth supplemented with 200 µg/ml Ampicillin. Cells were grown at 37°C to O.D.600=0.4, at which the point temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning, cells were collected by centrifugation and resuspended in 100 ml 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer/1 M NaCl/5 mM DTT/ 30 mM Spermidine/5 mM EDTA. Cells were lysed by two passages through the French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min. Ammonium sulfate was added to the supernatant to a final concentration of 0.3 g/ml. The resulting protein pellet was resuspended and dialyzed against buffer A containing 50 mM NaCl. The dialyzed protein (300 mg) was loaded onto a 45 ml FFQ- Sepharose column and eluted with a linear gradient from 50 - 500 mM NaCl. The peak fractions (16-30) were combined, dialyzed against buffer A containing 50 mM NaCl, loaded onto a 25 ml EAH-Sepharose column, and eluted with a linear gradient of 50-500 mM NaCl. The fractions containing the least contaminants were combined into two pools (pool I 10-17, pool II 19-27). Each pool was further purified on a 8 ml MonoQ column (performed under conditions described for the FFQ column above). The final beta subunit preparation is shown in the lane marked β of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 48 mg.

Example 27 - Identification of the *S. pyogenes* polC Gene and Purification of the Alpha-Large Polymerase Subunit

A search of the *B. subtilis* genome database with the *E. coli* alpha subunit amino acid sequence revealed two DNA sequences with a high scoring match which predicted two genes encoding alpha-like polymerase subunits. The DNA sequence with the second highest scoring match which encoded the largest of the two polymerase subunits also appeared to encode for the epsilon exonuclease domain at the N- terminus of the putative alpha subunit. A search of the *B. subtilis* genome database with *S. pyogenes* DNA sequence confirmed this nucleotide sequence to encode the Gram positive homolog of the *E. coli* replicative polymerase subunit (alpha). This Gram negative alpha-like subunit lacked homology to epsilon. The gene encoding the large alpha polypeptide sequence (alpha-large) will be referred to as

the product of the *polC* gene and the gene encoding the smaller Gram-negative alpha-like polymerase (alpha-small) will be referred to as the product of the *polE* or *dnaE* gene (see Example 28).

The alpha-large polymerase polypeptide is a product of two overlapping contigs; contig #197 (renamed #193) encodes the N-terminal 630 amino acids, and contig #278 (renamed #273) encodes the C-terminal 1392 amino acids. The putative Open Reading Frame generates a 1464 amino acid polypeptide (SEQ. ID. No. 18). Since the *polC* nucleotide sequence contained several NdeI sites, a primer was designed to mutate two restriction endonuclease sites in the pET11a nucleotide sequence upstream of the N-terminus of the gene; an XbaI restriction site was mutated to an NheI restriction site and an NdeI restriction site at the starting ATG was removed. A 74mer primer which spans from mutated XbaI site upstream of T7 promoter includes NheI site, rbs site (ribosome binding site), mutated NdeI site and first 10 amino acid codons of *polC* gene sequence. The following primers were used in a PCR reaction to amplify *polC* gene from *S. pyogenes* genomic DNA:

polC Upstream (SEQ. ID. No. 77)

```
ggataacaat tccccgctag caataatttt gtttaacttt aagaaggaga tatacccatg 60
tcagatttat tcgc 74
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polC Downstream (SEQ. ID. No. 78)

```
cgggtgtctct atctaaatga ctcatattggg atcctcgctt tatacgggtat gtcacag 57
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Elongase (BRL) produced the best amplification results. PCR reaction conditions were: 5 µg genomic DNA, 20 ng of each primer, 1 ml Elongase, 60 µM each dNTP, in 100 µl Elongase reaction buffer for 1 min at 94°C, 1 min at 55°C, and 6 min at 60°C repeated for 40 cycles. The resulting 4000 bp PCR fragment was digested with NheI and BamHI, purified and ligated into the pET11a vector (digested with XbaI and BamHI) to produce pET11a.S.p.*polC*.

The pET11a.S.p.*polC* plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 24L LB broth supplemented with 200 µg/ml Ampicillin. Cells were grown at 37°C to OD₆₀₀=0.4 at which point temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning, cells

(12g) were collected by centrifugation and resuspended in 100 ml 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer/1 M NaCl/5mM DTT/30 mM Spermidine/5 mM EDTA. Cells were lysed by two passages through the French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min. Ammonium sulfate was added to the supernatant to a final concentration of 0.226 g/ml. The precipitate was collected by centrifugation. The protein pellet (220 mg resuspended in buffer A) was dialyzed against buffer A containing 150 mM NaCl, loaded onto an 8 ml FFQ column equilibrated with buffer A containing 150 mM NaCl, and eluted with a linear gradient of buffer A containing 150-600mM NaCl. The fractions containing the least contaminants (fr# 42-64) were combined and precipitated with ammonium sulfate (0.226 g/ml). The precipitate was collected by centrifugation and resuspended in buffer A (10 mg/ml in 5 ml). A fraction (1 ml=10mgs) of the concentrated protein was dialyzed, loaded onto 10 ml ssDNA-agarose column, and eluted with a linear gradient of 50-500 mM NaCl. The peak fractions (fr# 30-50) were combined and concentrated with ammonium sulfate (as above). The final alpha-large subunit preparation is shown in lane marked α_L of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield= 4 mgs.

Example 28 - Identification of the *S. pyogenes* *dnaE* Gene and Purification of the Alpha-Small Polymerase

A search of the *B. subtilis* genome database using the *E. coli* alpha subunit amino acid sequence revealed two DNA sequences with a high scoring match which predicted two genes encoding for alpha-like polymerase subunits. The DNA sequence with the highest scoring match encodes a smaller alpha polymerase which does not contain an exonuclease domain. The putative short alpha DNA sequence is a product of the open reading frame in contig #253 of the *S. pyogenes* genome database. A set of PCR primers to 5'- and 3'-termini of the putative gene sequence were designed to include restriction enzyme recognition sequences for NdeI and BamHI, respectively. The primers were:

α -short Upstream (SEQ. ID. No. 79)

gggaacaaga taaccaagga ggaacccatg gttgctcaac ttg

α -short Downstream (SEQ. ID. No. 80)

cgaatagcag cgttcataacc aggatcctcg cgcgcactgg

40

5 A PCR product obtained with these primers was digested with NdeI and BamHI, purified and ligated into pET11a vector to produce pET11a.S.p.dnaE.

The pET11a.S.p.dnaE plasmid was transformed into the BL21(DE3)RecA- strain of E. coli. A single colony from an overnight transformation was used to inoculate 12L LB broth supplemented with 200 μ g/ml Ampicillin. Cells were grown at 37°C to OD₆₀₀=0.4, at which point temperature was lowered to 15°C and 0.5 mM IPTG was added. Induction proceeded for 16 hrs. In the morning, cells were collected by centrifugation and resuspended in 100 mls 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer/5 mM DTT/30 mM Spermidine/1M NaCl/5 mM EDTA. Cells were lysed by two passages through the French press (15,000 psi), followed by centrifugation at 13,000 rpm for 30 min. Ammonium sulfate was added to the supernatant to a final concentration of 0.226 g/ml. The precipitate was collected by centrifugation. The protein pellet (resuspended in buffer A) was then dialyzed against buffer A. The dialyzed protein (600 mg) was loaded onto a 30 ml FFQ and eluted with a linear gradient of buffer A containing 50-500 mM NaCl. The peak fractions (200 mg in fr # 70-79) were dialyzed and loaded onto a 100 ml Heparin-Affigel column. The fractions containing the least contaminants (100 mg from fr # 18-30) were pooled and dialyzed against buffer A containing 300 mM NaCl. The dialysate (50 mg) was loaded onto a 50 ml ssDNA-agarose column and eluted with a linear gradient of 300mM - 1M NaCl. The final alpha-small subunit preparation is shown in lane marked α_s of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 25 mg.

Example 29 - Identification of the *S. pyogenes* ssb Gene and Purification of the Single Strand DNA-Binding Protein

30 Search of the *S. pyogenes* genome using the *B. subtilis* SSB amino acid sequence identified a polypeptide in contig #230(212) as having highest homology to single strand binding protein of several Gram negative bacteria. This contig lacked the first 26 amino acids at the N-terminus. Circular PCR was employed to identify the DNA encoding the N-terminus of the putative SSB protein. *S. pyogenes* genomic

DNA was digested overnight with ApoI (5 µg chromosomal DNA in a 50 µl reaction). The DNA was extracted with phenol and precipitated with ethanol. The ApoI digested chromosomal DNA was self-ligated to generate circular template for future use in the circular PCR. A circular PCR was performed with primers designed to anneal back-to-back to amplify circularized ApoI reaction fragments. The primers were:

ssb.circ Upstream (SEQ. ID. No. 81)

accatttttg cttttaaagg tacggttaac agcaagtgtg aaggtagcc 49

ssb.circ Downstream (SEQ. ID. No. 82)

gaacgcgagg cagatttcat taactgtgtg atctggcg 38

The PCR reaction conditions were as follows: 100 ng circularized *S. pyogenes* genomic DNA, 20 ng each primer, 1 ml Elongase, 60 µM each dNTP, 100 U Elongase reaction buffer. Amplification was performed for 40 cycles as follows: denature, 1 min at 94°C; anneal, 1 min at 55°C; and extend, 5 min at 68°C. PCR products were cloned into the Topo TA vector following instructions of the manufacturer (Promega). Several positive clones were sequenced to obtain N-terminal nucleotide sequence. This information lead to design of the following primers with which the use of a standard PCR reaction generated whole *ssb* gene products. The primers were:

ssb Upstream (SEQ. ID. No. 83)

tttaaaagag ggtagcatat gattaataat gtagtactag ttggtcgc 48

ssb Downstream (SEQ. ID. No. 84)

tttaaattta aacctagggt caatccattc tgactagaat ggaagatcgt c 51

The resulting PCR product was digested with NdeI and BamHI, purified and ligated into pET11a vector to produce pET11a.S.p. ssb.

The pET11a.S.p.ssb plasmid was transformed into the BL21(DE3)RecA- strain of *E. coli*. A single colony from an overnight transformation was used to inoculate 12L LB broth supplemented with 200 µg/ml Ampicillin. Cells

were grown at 37°C to OD₆₀₀=0.5, at which point 0.5 mM IPTG was added. At the end of the 3 hr induction, cells were collected by centrifugation and resuspended in 100 ml of 50 mM Tris-HCl (pH 7.5)/ 10% Sucrose /1X Heat Lysis Buffer/5 mM DTT/5 mM EDTA. The cell lysis was initiated upon addition of 0.4 mg/ml lysozyme followed by a 1 hr incubation on ice. The lysate was clarified by centrifugation at 13,000 rpm for 30 min. The SSB protein was significantly purified by sequential fractionation with ammonium sulfate in the following manner. Solid ammonium sulfate was added to the clarified lysate to a final concentration of 0.24 g/ml and the precipitated protein was collected by centrifugation at 13,000 rpm for 30 min. The resulting pellet was homogenized in buffer A(0.1 M NaCl) + 0.24 g/ml ammonium sulfate and the precipitate was collected by centrifugation. This procedure was repeated with buffer A(0.1 M NaCl) + 0.2 g/ml ammonium sulfate, buffer A(0.1 M NaCl + 0.15 g/ml ammonium sulfate, and buffer A(0.1 M NaCl) + 0.13 g/ml ammonium sulfate. The final pellet was resuspended in buffer A + 0.15 M NaCl and dialyzed against the same buffer. The resulting pellet was resuspended in buffer A and dialyzed against buffer A containing 500 mM NaCl. The dialysate (300 mg) was diluted to 0.15 M NaCl before it was loaded onto a 20 ml MonoQ column and eluted with a linear gradient of 0.15 M - 0.5 M NaCl in buffer A. The SSB protein elutes in the very beginning of the gradient. The peak fractions were combined (150 mg in fractions 16-30), diluted to 0.05 M NaCl, loaded onto a 10 ml ssDNA-agarose column, and eluted with 0.5 M NaCl. The peak fractions (32-62) were combined and frozen. The SSB was further purified over a MonoQ column to remove contaminating polymerase activity. The final single strand DNA binding protein preparation is shown in lane marked ssb of the Coomassie Blue stained SDS-polyacrylamide gel of Figure 14. Yield = 120 mg.

Example 30 - First Demonstration that *S. pyogene hola* Encodes a Delta Subunit Involved In Replication: Assembly of $\tau\delta\delta'$ Complex

Gel filtration is a standard analytical technique to demonstrate direct protein-protein interaction. Purified τ , δ , δ' proteins were used to examine whether they form a protein complex assembly. Gel filtration of τ mixed with either δ , δ' , or both δ and δ' was performed using an HR 10/30 Superose 6 column equilibrated with

buffer A containing 100 mM NaCl. Either δ (200 μ g), δ' (200 μ g), or a mixture of δ and δ' (200 μ g each) was incubated for 30 min at 15°C in 100 μ l of buffer A containing 100 mM NaCl, and the entire mixture was injected onto the column. The mixture was resolved on the column by collection of 170 μ l fractions after the initial void (6.6 μ l) volume was collected. Fractions were analyzed by 10% SDS-polyacrylamide gels (30 μ l/lane) stained with Coomassie Blue.

The results, in Figure 15, demonstrate that under these conditions the τ protein exhibits no (weak) interaction with the delta (Figure 15B) and the delta prime subunits (Figure 15C) individually, and yet assembles readily into a complex when all the subunits are mixed in the reaction (Figure 15A). The τ protein was mixed with a 2-fold molar excess of each δ and δ' , then gel filtered. A complex of $\tau\delta\delta'$ was formed as demonstrated by coelution of δ and δ' with τ (fr# 22-30) whereas excess $\delta\delta'$ complex elutes in later fractions (fr#38-46). To determine whether individual δ or δ' subunits interact with τ , the τ subunit was mixed with either δ or δ' and then gel filtered. The results demonstrate that a gel filterable complex does not form when τ is mixed with δ (Figure 15B) or δ' (Figure 15C) subunits individually, as indicated by the absence of these subunits in the τ containing fractions (fr#20-26). Therefore, it appears that the presence of both δ and δ' subunits is essential for the formation of the $\tau\delta\delta'$ complex.

Example 31 - Second Demonstration that *S. pyogenes holA* Encodes Delta: Functional Assembly of β on DNA

Gel filtration was used to demonstrate that the τ , δ , δ' proteins form a functional clamp loading complex which is able to load the β clamp onto a circular DNA molecule. The reaction contained 0.5 pmol of gp2 nicked pBluescript plasmid (a circular double strand plasmid with a single nick produced by M13 gp2 protein), 1 pmol [32 P] β , 0.5 pmol $\tau\delta\delta'$ complex, 0.25 pmol of either δ , δ' , τ were used in individual experiments when a subassembly of the complex was tested ($\tau\delta$, $\tau\delta'$, $\delta\delta'$) in 75 μ l buffer B (20 mM Tris-HCl (pH 7.5), 20 % glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM $MgCl_2$). β was incubated with nicked DNA for 10 min at 37°C either alone, or in combination with various assemblies of the τ complex. All gel

filtration experiments were performed at 4°C. The reaction mixtures were applied to a 5 ml column of Bio-Gel 15M (Bio-Rad) equilibrated in buffer B containing 100 mM NaCl. Fractions of 170 µl were collected and quantitated in the Scintillation counter.

The results, in Figure 16, demonstrate that the assembly of the ring onto a circular DNA molecule requires the presence of τ , δ , and δ' proteins (Figure 16A). In absence of any one of the subunits, loading onto DNA does not occur (Figure 16B-E). The clamp loader complex ($\tau\delta\delta'$) can be supplied as a mixture of τ , δ , δ' subunits or as an assembled complex (purified from unassembled subunits by gel filtration, or by ion exchange chromatography on MonoQ). Proteins bound to the large DNA molecule elute in the early fractions (void fr# 10-17) and resolve from free proteins that elute in later fractions (fr# 18-35).

Example 32 - The τ Subunit Product of the *dnaX* Gene Binds α -large

The interaction of *S. pyogenes* α and τ proteins was examined by analyzing a mixture of the proteins by gel filtration. Gel filtration of τ , α -large or a mixture of α -large and τ was performed using an HR 10/30 Superose 6 column equilibrated with buffer A containing 100 mM NaCl. Either α -large (400 µg) (200 µM) or a mixture of α -large and τ was incubated for 30 min at 15°C in 100 µl of buffer A containing 100 mM NaCl, and the entire mixture was injected onto the column. The mixture was resolved on the column by collection of 170 µl fractions after the initial void (6.6 ml) volume was collected. Fractions were analyzed by 10% SDS-polyacrylamide gels (30 µl/lane) stained with Coomassie Blue.

The results show a complex of $\alpha_L\tau$ was formed as demonstrated by coelution of α -large and τ (fr# 30-38) proteins (Figure 17A) compared to the elution profile of individual proteins (Figure 17B-C). Also, the migration of the τ in the $\alpha_L\tau$ complex changes significantly to a larger complex (4 fractions, from fr# 37 to fr# 33).

Example 33 - Formation of $\alpha_L\tau\delta\delta'$ Complex

To determine whether a $\alpha_L\tau\delta\delta'$ complex could form, the following components were mixed: α -large (400 µg, 2.5 nmol), τ (200 µg, 1.3 nmol), δ (200 µg,

4.8 nmol), δ' (200 μ g, 5.75 pmol) in a final volume of 150 μ l. The mixture was diluted to 300 μ l with buffer A to lower conductivity of the sample to that equivalent of 100 mM NaCl and incubated for 30 min at 15°C. The mixture was injected onto a Superose 6 column (equilibrated with buffer A containing 100 mM NaCl) and fractions (170 μ l) were collected after an initial 6.6 ml of void volume was collected. Fractions were analyzed by 10% SDS-polyacrylamide gels (30 μ l/lane) stained with Coomassie Blue.

A gel filterable complex (Figure 18A) of $\alpha_L\tau\delta\delta'$ was formed as demonstrated by coelution of τ , δ and δ' with α -large (fr# 14-26), whereas excess $\delta\delta'$ complex elutes in later fractions (fr# 30-38). The migration of the $\tau\delta\delta'$ protein complex in the $\alpha_L\tau\delta\delta'$ complex does not change significantly. The complex might dissociate under the nonequilibrium conditions of gel filtration due to low concentration of proteins, salt concentration and speed of resolution.

Next, ion exchange chromatography was used to analyze the protein mixture to prepare the reconstituted $\alpha_L\tau\delta\delta'$ complex of *S. pyogenes*. The $\alpha_L\tau\delta\delta'$ complex was reconstituted upon mixing α -large (10 mg, 62 nmol), τ (6 mg, 72 nmol), δ (3.3 mg, 80 nmol), δ' (1.6 mg, 90 nmol). The α , τ , δ , δ' protein mixture was dialyzed for 2 hrs against buffer A containing 50 mM NaCl. The entire mixture was loaded onto a 1 ml MonoQ column equilibrated in buffer A containing 50 mM NaCl. Proteins were eluted with a 20 column volume linear gradient of 50-500 mM NaCl in buffer A and 0.25 ml fractions were collected. Fractions were analyzed by 10% SDS-polyacrylamide gels (20 μ l/lane) stained with Coomassie Blue.

Generally, the reconstitution of the $\alpha_L\tau\delta\delta'$ complex on a MonoQ column results in a tight salt resistant complex (Figure 18B, fr# 23-35) which elutes at 500 mM NaCl. The high concentration of the proteins in the eluted fractions contributes to stability of the complex.

Example 34 - The *S. pyogenes* Three Component Pol III-L Polymerase Is Rapid and Processive In DNA Synthesis

It was previously demonstrated (i.e., in Examples 29 and 30) that the putative delta subunit plays an integral part in the assembly of the $\tau\delta\delta'$ complex

(Figure 15) and that this complex is sufficient to assemble β clamps onto circular primed DNA (Figure 16). It was also shown that the strong interaction between the α - large and τ subunits (Figure 17) results in an isolatable $\alpha_L\tau\delta\delta'$ complex (Figure 18), similar to that of the *E. coli* DNA polymerase III*.

5 The MonoQ fractions containing $\alpha_L\tau\delta\delta'$ complex were then used to assemble β onto primed DNA and determine whether this now resulted in rapid and processive DNA synthesis. Replication reactions contained 70 ng of singly primed M13mp18 ssDNA and 0.82 μ g of *S. pyogenes* SSB in 25 μ l buffer C (20 mM Tris-HCl (pH 7.5), 4 % glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM $MgCl_2$)
10 with 60 μ M each of dGTP, dCTP, and dATP, 30 μ M cold TTP and 20 μ M [α - 32 P] TTP (specific activity of 2,000-4,000 cpm/pmol). The complex is assembled onto DNA in the following manner: 40 ng (3:1) or 140 ng (10:1) of the $\alpha_L\tau\delta\delta'$ complex and 60 ng of β protein were preincubated for 2 min at 30°C in presence of SSB coated primed M13 DNA and two nucleotides (dCTP and dGTP). Reactions were initiated by
15 addition of the two remaining nucleotides dATP and TTP and quenched with an equal volume of 1% SDS/40 mM EDTA. Each time point is a separate reaction.

 A time course of replication on singly primed circular M13mp18 ssDNA is shown in Figure 19. The agarose gel analysis shows conversion of the oligonucleotide primed single stranded DNA to the slower migrating replicative form
20 II. The fact that the speed of synthesis is independent of the concentration of polymerase in the reaction indicates that the $\alpha_L\tau\delta\delta'$ complex synthesizes DNA in a rapid and a highly processive manner. The *S. pyogenes* $\alpha_L\tau\delta\delta'$ complex in presence of the β clamp, completely replicates (is able to complete replication of) 7250 nt of M13mp18 ssDNA in 8-9 sec.

25 **Example 35 - The *S. pyogenes* DnaE (α -small) Forms a Three-Component Polymerase with $\tau\delta\delta'$ and β**

 The *S. pyogenes* DnaE (α -small) polymerase is more homologous to *E. coli* α
30 than *S. pyogenes* PolC. Thus, it seems reasonable to expect that the DnaE polymerase may also function with the β clamp (Figs. 21A-B). To test DnaE for function with $\tau\delta\delta'$ and β , replication reactions contained 70 ng (25 fmol) of 30-mer singly primed

M13mp18 ssDNA, 0.82 μ g of *S. pyogenes* SSB, and 3.3 ng - 300 ng of DnaE (25 fmol - 2.3 pmol) in 23.5 μ l of 20 mM Tris-HCl (pH 7.5), 4% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol (DTT), 40 μ g/ml BSA, 2 mM ATP, 8 mM $MgCl_2$, and 60 μ M each of dGTP and dCTP. When present, reactions included 43.3 ng of β and 10 ng of $\tau\delta\delta'$. Reactions were preincubated for 3 min at 37°C, and then NaCl was added to 40 mM followed by another 2 min at 37°C. DNA synthesis was initiated upon addition of 1.5 μ l of 1.5 mM dATP, 0.5 mM [$\alpha^{32}P$]-dTTP (specific activity 2,000-4,000 cpm/pmol). Aliquots of 25 μ l were removed at the indicated times and quenched with an equal volume (25 μ l) of 1% SDS, 40 mM EDTA. One-half of the quenched reaction was analyzed for total deoxynucleotide incorporation using DE81 filter paper and the other half was analyzed on a 0.8% neutral agarose gel. The effect of TMAU was also examined, in which 100 μ M TMAU in DMSO (2% DMSO final concentration) was present. In this case, replication was allowed to proceed for 1 min before being quenched with 25 μ l of 1% SDS, 40 mM EDTA.

At a saturating concentration of DnaE polymerase, the time course of primer extension shows that it completes an M13mp18 primed ssDNA template within 2 minutes for a speed of at least 60 nucleotides/s (Fig. 21C). This rate of synthesis holds true for the highest amount of DnaE in the rightmost panel of the figure. As the DnaE concentration is decreased, a longer time is required to complete the circular template, indicating that the DnaE polymerase is not processive over the entire length of the M13mp18 template. If the DnaE polymerase were fully processive during synthesis of the 7.2 kb ssDNA circle, the product profile over time would be qualitatively similar at all concentrations of enzyme, but the overall intensity of the profile would be diminished. This particular experiment was performed in the absence of β , but presence of $\tau\delta\delta'$. When repeated in the presence of β but without $\tau\delta\delta'$, and in the absence of both β and $\tau\delta\delta'$, results similar to those shown in Fig. 21C were observed.

In the presence of β and $\tau\delta\delta'$, DnaE polymerase is stimulated in synthesis at low concentration, indicating that β increases the processivity and/or speed of DnaE (Figs. 21C-D). At higher concentrations of DnaE, the presence of $\beta/\tau\delta\delta'$ has no effect on the rate of synthesis, and thus β does not increase the intrinsic speed of the enzyme (i.e., panels 3 and 4 of Fig. 21D). Hence, the effect of the β clamp on DnaE is

primarily due to an increase in processivity. The profile of product length over time remains essentially unchanged at the different DnaE concentrations, and therefore the processivity of DnaE, with β is at least equal to the 7.2 kb length of the M13mp18 substrate.

5 The DnaE sequence does not show homology to an exonuclease, implying that it may have no associated nuclease activity. The DnaE preparation was examined for the presence of a 3'-5' exonuclease (Fig. 21E). The DnaE and PolC polymerases were each incubated with a 5' ³²P-labeled oligonucleotide, followed by analysis in a sequencing gel. The result showed no degradation of the oligonucleotide by DnaE.
10 PolC is a known 3'-5' exonuclease and it digests the end-labeled oligonucleotide as expected.

 Gram positive PolC is known to be inhibited by the antibiotic hydroxyphenylaza-uracil ("HPUra") and its derivatives. In Fig. 21F, the PolC- $\tau\delta\delta'$, β and DnaE were tested for inhibition of synthesis on SSB coated primed M13mp18 ssDNA by an HPUra derivative, trimethylanilino-uracil ("TMAU"). The PolC- $\tau\delta\delta'$ β
15 enzyme was prevented from forming the RFII product by TMAU. In contrast, the DnaE polymerase was not affected by TMAU in the presence of $\tau\delta\delta'/\beta$ (nor in the absence of $\tau\delta\delta'/\beta$, not shown).

20 Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. An isolated DNA molecule from a Gram positive bacterium, the isolated DNA molecule comprising a coding region from a *polC* gene, a *dnaE* gene, a *hola* gene, a *holB* gene, a *dnaX* gene, a *dnaN* gene, a *ssb* gene, a *dnaG* gene, or a *dnaB* gene.

2. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *polC* gene.

3. The isolated DNA molecule according to claim 2, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

4. An isolated DNA molecule according to claim 3, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 18.

5. The isolated DNA molecule according to claim 4, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 17.

6. The isolated DNA molecule according to claim 2, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 17 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

7. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *dnaE* gene.

8. The isolated DNA molecule according to claim 7, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

9. The isolated DNA molecule according to claim 8, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 20.

10. The isolated DNA molecule according to claim 9, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 19.

5 11. The isolated DNA molecule according to claim 7, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 19 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

10 12. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *hola* gene.

13. The isolated DNA molecule according to claim 12, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

15 14. The isolated DNA molecule according to claim 13, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 22.

20 15. The isolated DNA molecule according to claim 14, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 21.

25 16. The isolated DNA molecule according to claim 12, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 21 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

17. The isolated DNA molecule according to claim 12, wherein the Gram positive bacterium is *Staphylococcus aureus*.

30 18. The isolated DNA molecule according to claim 17, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 12.

19. The isolated DNA molecule according to claim 18, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 11.

20. The isolated DNA molecule according to claim 12, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 11 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M
5 SSC buffer at a temperature of 37°C.

21. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *holB* gene.

10 22. The isolated DNA molecule according to claim 21, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

23. The isolated DNA molecule according to claim 22, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 24.
15

24. The isolated DNA molecule according to claim 23, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 23.

25. The isolated DNA molecule according to claim 21, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 23 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M
20 SSC buffer at a temperature of 37°C.

26. The isolated DNA molecule according to claim 21, wherein the Gram positive bacterium is *Staphylococcus aureus*.
25

27. The isolated DNA molecule according to claim 26, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 14.

30 28. The isolated DNA molecule according to claim 27, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 13.

29. The isolated DNA molecule according to claim 21, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 13 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

5

30. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *dnaX* gene.

31. The isolated DNA molecule according to claim 30, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

10

32. The isolated DNA molecule according to claim 31, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 26.

15

33. The isolated DNA molecule according to claim 32, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 25.

34. The isolated DNA molecule according to claim 30, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 25 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

20

35. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *dnaN* gene.

25

36. The isolated DNA molecule according to claim 35, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

37. The isolated DNA molecule according to claim 36, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 28.

30

38. The isolated DNA molecule according to claim 37, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 27.

39. The isolated DNA molecule according to claim 35, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 27 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

40. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *ssb* gene.

41. The isolated DNA molecule according to claim 40, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

42. The isolated DNA molecule according to claim 41, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 30.

43. The isolated DNA molecule according to claim 42, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 29.

44. The isolated DNA molecule according to claim 40, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 29 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

45. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *dnaG* gene.

46. The isolated DNA molecule according to claim 45, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

47. The isolated DNA molecule according to claim 46, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 32.

48. The isolated DNA molecule according to claim 47, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 31.

49. The isolated DNA molecule according to claim 45, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 31 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

50. The isolated DNA molecule according to claim 1, wherein the DNA molecule comprises the coding region from the *dnaB* gene.

51. The isolated DNA molecule according to claim 50, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

52. The isolated DNA molecule according to claim 51, wherein the DNA molecule encodes an amino acid sequence comprising SEQ. ID. No. 34.

53. The isolated DNA molecule according to claim 52, wherein the DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 33.

54. The isolated DNA molecule according to claim 50, wherein the DNA molecule hybridizes to a nucleic acid molecule of SEQ. ID. No. 33 under stringent conditions characterized by use of a hybridization buffer comprising 0.9M SSC buffer at a temperature of 37°C.

55. An expression system comprising an expression vector into which is inserted a heterologous DNA molecule according to claim 1.

56. The expression system according to claim 55, wherein the heterologous DNA molecule is in sense orientation and correct reading frame.

57. A host cell comprising a heterologous DNA molecule according to claim 1.

58. An isolated protein or polypeptide from a Gram positive bacterium, wherein the isolated protein or polypeptide is alpha-large, alpha-small, delta, delta prime, tau, beta, SSB, DnaG, or DnaB.

5

59. The isolated protein or polypeptide according to claim 58, wherein the isolated protein or polypeptide is alpha-large.

60. The isolated protein or polypeptide according to claim 59, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

10

61. The isolated protein or polypeptide according to claim 60, wherein the alpha-large protein or polypeptide comprises an amino acid sequence of SEQ. ID. No. 18.

15

62. The isolated protein or polypeptide according to claim 58, wherein the isolated protein or polypeptide is alpha-small.

63. The isolated protein or polypeptide according to claim 62, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

20

64. The isolated protein or polypeptide according to claim 63, wherein the alpha-small protein or polypeptide comprises an amino acid sequence of SEQ. ID. No. 20.

25

65. The isolated protein or polypeptide according to claim 58, wherein the isolated protein or polypeptide is delta.

66. The isolated protein or polypeptide according to claim 65, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

30

67. The isolated protein or polypeptide according to claim 66,
wherein the delta protein or polypeptide comprises an amino acid sequence of SEQ.
ID. No. 22.

5 68. The isolated protein or polypeptide according to claim 65,
wherein the Gram positive bacterium is *Staphylococcus aureus*.

69. The isolated protein or polypeptide according to claim 68,
wherein the delta protein or polypeptide comprises an amino acid sequence of SEQ.
10 ID. No. 12.

70. The isolated protein or polypeptide according to claim 58,
wherein the isolated protein or polypeptide is delta prime.

15 71. The isolated protein or polypeptide according to claim 70,
wherein the Gram positive bacterium is *Streptococcus pyogenes*.

72. The isolated protein or polypeptide according to claim 71,
wherein the delta prime protein or polypeptide comprises an amino acid sequence of
20 SEQ. ID. No. 24.

73. The isolated protein or polypeptide according to claim 70,
wherein the Gram positive bacterium is *Staphylococcus aureus*.

25 74. The isolated protein or polypeptide according to claim 73,
wherein the delta prime protein or polypeptide comprises an amino acid sequence of
SEQ. ID. No. 14.

75. The isolated protein or polypeptide according to claim 58,
30 wherein the isolated protein or polypeptide is tau.

76. The isolated protein or polypeptide according to claim 75,
wherein the Gram positive bacterium is *Streptococcus pyogenes*.

77. The isolated protein or polypeptide according to claim 76,
wherein the tau protein or polypeptide comprises an amino acid sequence of SEQ. ID.
No. 26.

5

78. The isolated protein or polypeptide according to claim 58,
wherein the isolated protein or polypeptide is beta.

10

79. The isolated protein or polypeptide according to claim 78,
wherein the Gram positive bacterium is *Streptococcus pyogenes*.

15

80. The isolated protein or polypeptide according to claim 79,
wherein the beta protein or polypeptide comprises an amino acid sequence of SEQ.
ID. No. 28.

81. The isolated protein or polypeptide according to claim 58,
wherein the isolated protein or polypeptide is SSB.

20

82. The isolated protein or polypeptide according to claim 81,
wherein the Gram positive bacterium is *Streptococcus pyogenes*.

83. The isolated protein or polypeptide according to claim 82,
wherein SSB comprises an amino acid sequence of SEQ. ID. No. 30.

25

84. The isolated protein or polypeptide according to claim 58,
wherein the isolated protein or polypeptide is DnaG.

30

85. The isolated protein or polypeptide according to claim 84,
wherein the Gram positive bacterium is *Streptococcus pyogenes*.

86. The isolated protein or polypeptide according to claim 85,
wherein the DnaG protein or polypeptide comprises an amino acid sequence of SEQ.
ID. No. 32.

87. The isolated protein or polypeptide according to claim 58, wherein the isolated protein or polypeptide is DnaB.

5 88. The isolated protein or polypeptide according to claim 87, wherein the Gram positive bacterium is *Streptococcus pyogenes*.

89. The isolated protein or polypeptide according to claim 88, wherein the DnaB protein or polypeptide comprises an amino acid sequence of SEQ.
10 ID. No. 34.

90. A method of identifying compounds which inhibit the activity of a polymerase product of *polC* or *dnaE* comprising:

15 forming a reaction mixture comprising a primed DNA molecule, a polymerase product of *polC* or *dnaE*, a candidate compound, a dNTP, and optionally either a beta subunit, a tau complex, or both the beta subunit and the tau complex, wherein at least one of the polymerase product of *polC* or *dnaE*, the beta subunit, the tau complex, or a subunit or combination of subunits thereof is derived from a Eubacteria other than *Escherichia coli*;

20 subjecting the reaction mixture to conditions effective to achieve nucleic acid polymerization in the absence of the candidate compound;

analyzing the reaction mixture for the presence or absence of nucleic acid polymerization extension products; and

25 identifying the candidate compound in the reaction mixture where there is an absence of nucleic acid polymerization extension products.

91. The method according to claim 90, wherein the polymerase product of *polC* or *dnaE* is from a *Streptococcus* bacterium or a *Staphylococcus* bacterium.

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International Bureau



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- (21) International Application Number: **PCT/US00/20666**
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- (71) Applicant (*for all designated States except US*): **THE ROCKEFELLER UNIVERSITY** [US/US]; Office of General Counsel, 1230 York Avenue, New York, NY 10021 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **O'DONNELL, Michael, E.** [US/US]; 16 Maple Lane, Hastings-on-Hudson, NY 10706 (US). **BRUCK, Irina** [US/US]; Apartment 11M, 1161 York Avenue, New York, NY 10021 (US). **ZHANG, Dan** [US/US]; 68-37 108th Street #3B, Forest Hills, NY 11375 (US).
- Published:**
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

(57) Abstract: The present invention relates to alpha-large, alpha-small, delta, delta prime, tau, beta, SSB, DnaG DnaB encoding genes from Gram positive bacterium, preferably *Streptococcus* and *Staphylococcus* bacterium. The formation of functional polymerase as well as the use of such a polymerase in sequencing and amplification is also disclosed. The individual genes and proteins or polypeptides are useful in identification of compounds with antibiotic activity.

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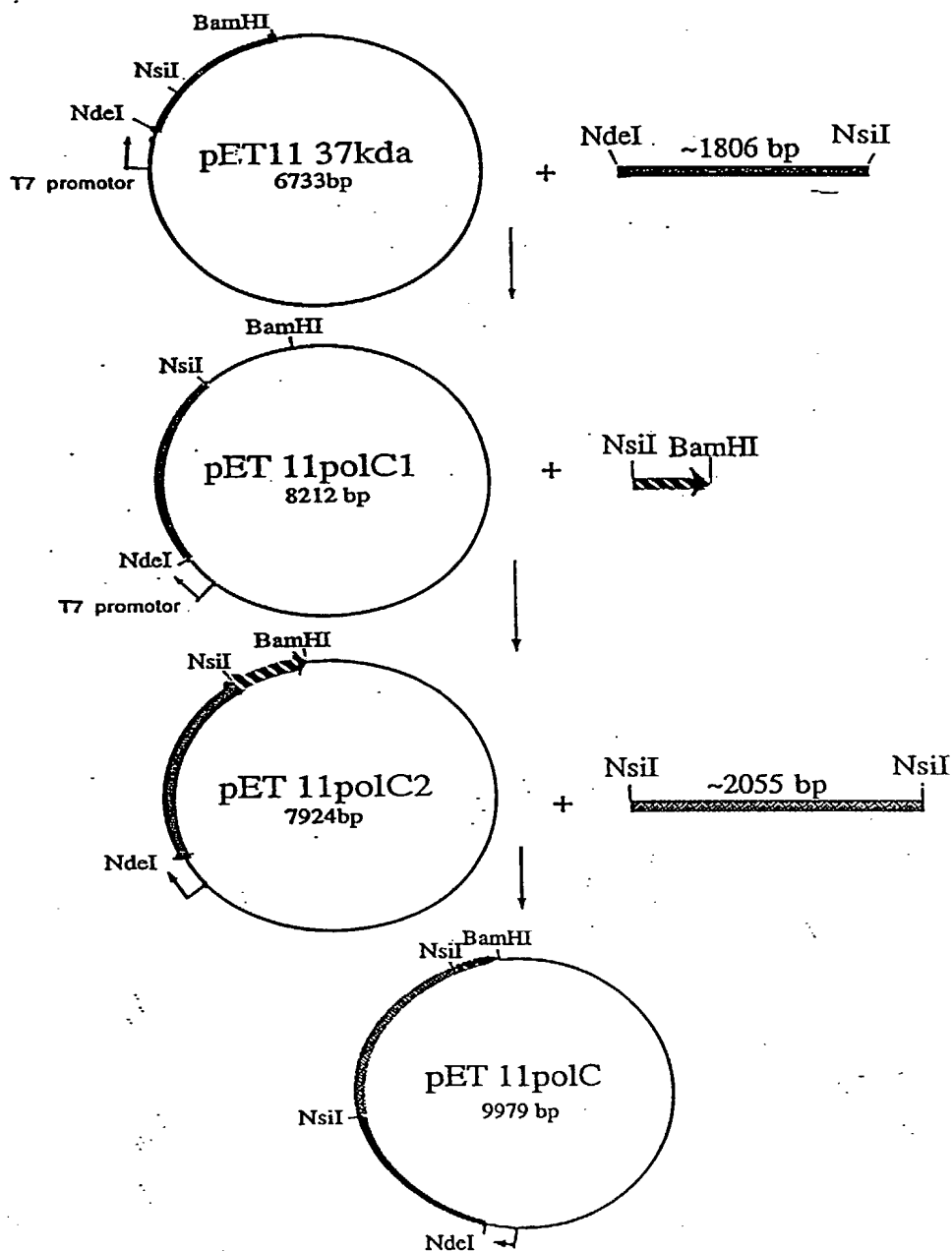


FIGURE 1

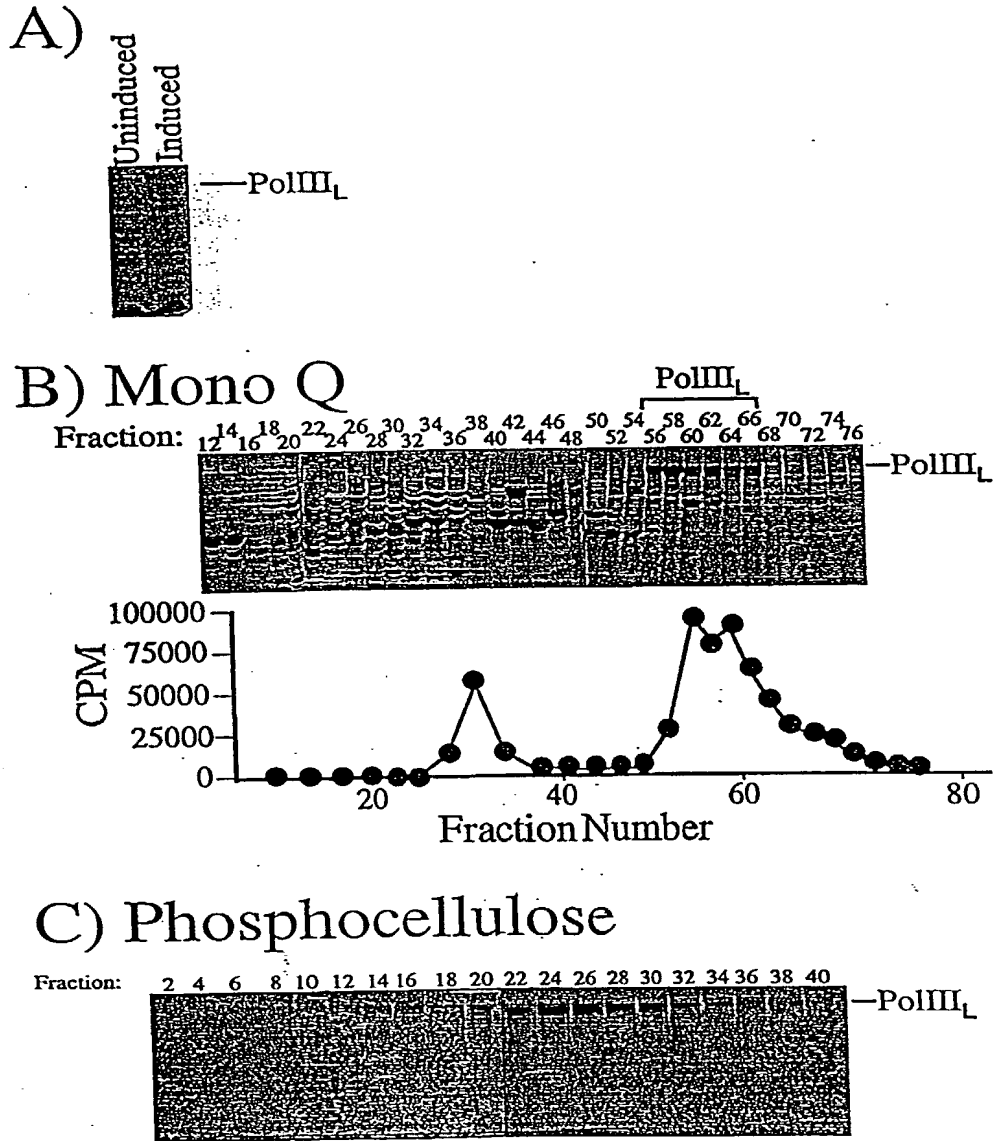


FIGURE 2

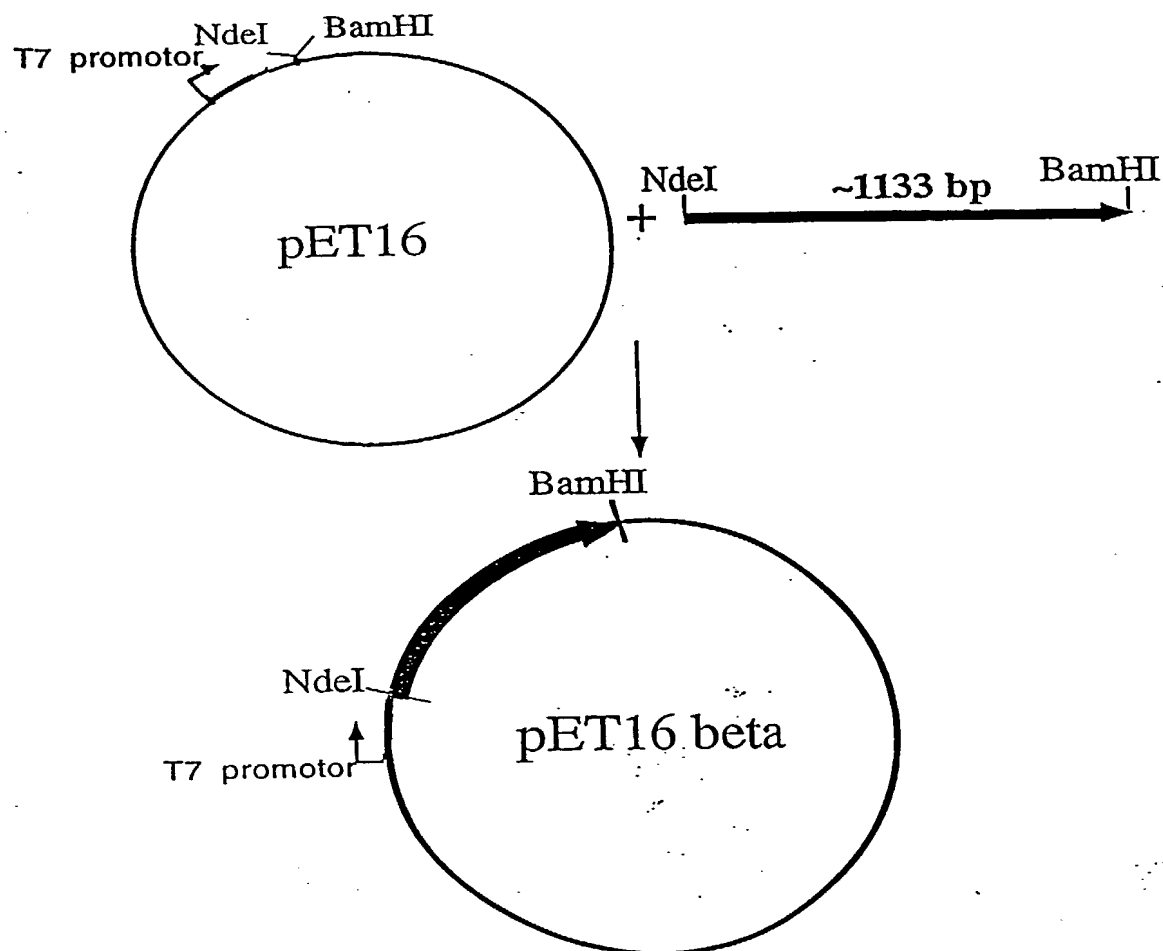
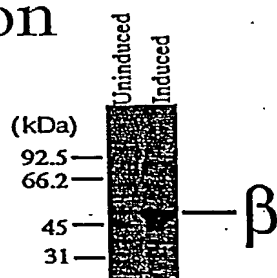
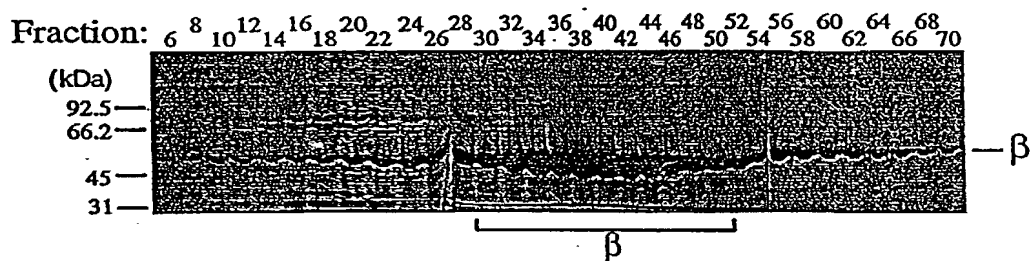


FIGURE 3

A) Induction



B) Nickel column



C) Mono Q

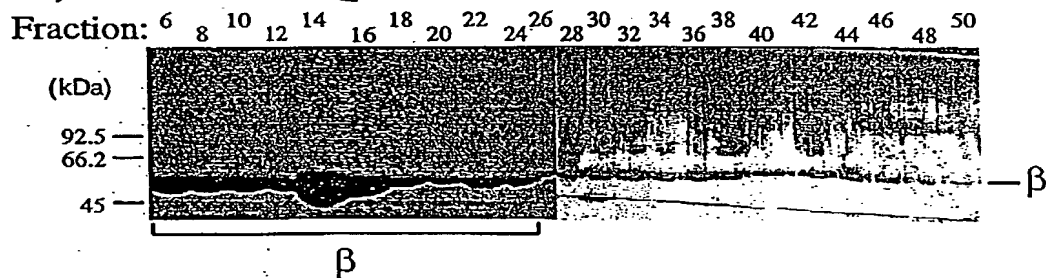
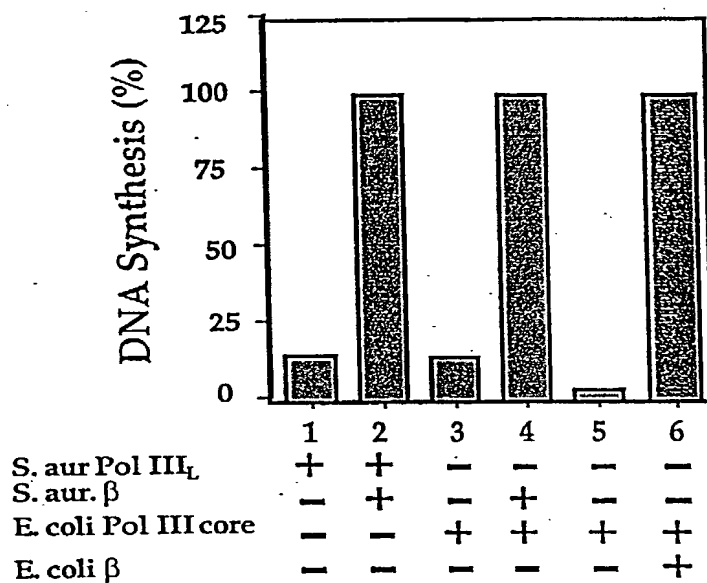


FIGURE 4

A) Linear DNA



B) Circular DNA

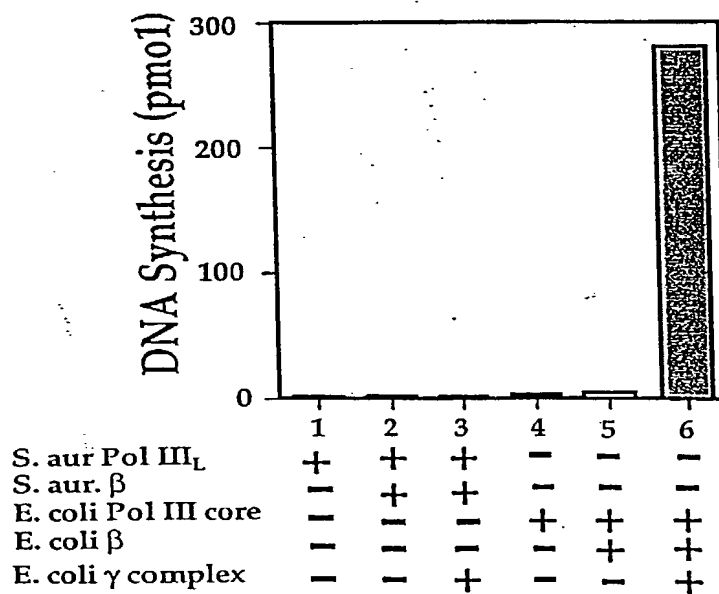


FIGURE 5

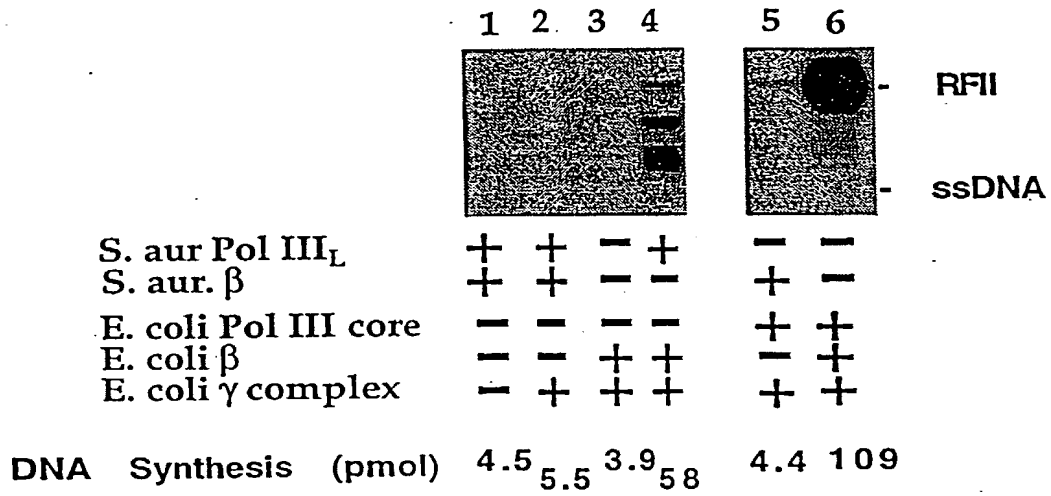
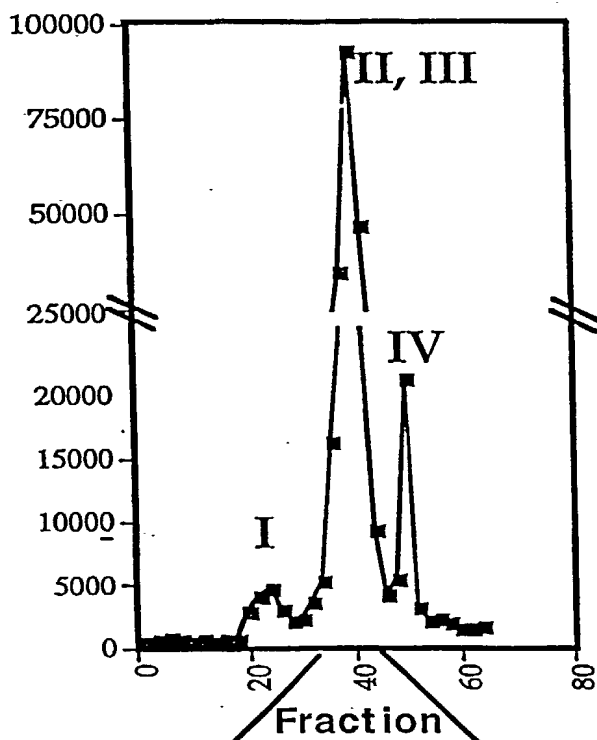


FIGURE 6

A) MonoQ



B) P-Cell

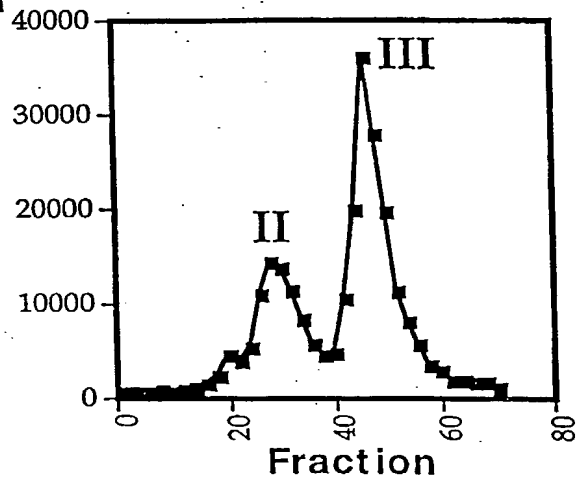
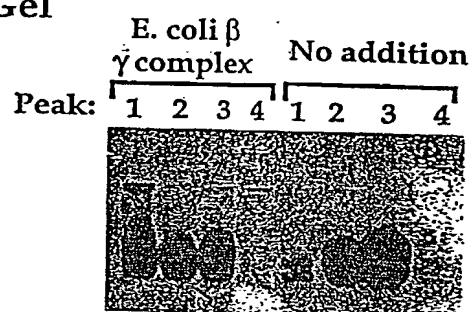


FIGURE 7

A) Agarose Gel



B) DNA Synthesis

Addition	DNA Synthesis (pmol)			
	PEAK			
	Peak 1	Peak 2	Peak 3	Peak 4
None	22.7	70.6	146.1	4.7
E. coli β , γ complex	72.9	61.2	71.4	25.9

FIGURE 8

[illegible]

FIGURE 9

		ATP site
S.aureus	MGYCLWRCNLDYQALFVVTP-KFEDVVGQEHSEDCAMG-----SHAYLFSGPRGTGKT	
B.sub.	-----MSYQALYRVFRPQRFEDVVGQEHITKTLQNALLOKFSHAYLFSGPRGTGKT	
E.coli	-----MSYQVLARKWRPQTFADVVGQEHVLTALANGLSLGRHHAYLFSGTRGVGKT	
	** * * * *	***** ** ***
	Zn++ finger	
S.aureus	SIKVFKAICLNSTDGEPNCNECHICKGITQGTNSDVIEIDAASNNGVDEIRNIRDKVYA	
B. sub	SAKIFAKAVNCEHAPVDEPCNECAACKGITNGSISDVIEIDAASNNGVDEIRDIRDKVKA	
E. coli	SIARLLAKGLNCETGITATPCGVCDNCREIEQGRFVDLIEIDAASRTKVEDTRDLLDNVQYA	
	* * * * *	***** ** * * *
S.aureus	PSESKYKVYIIDEVHMLTTGAFNALLKTLLEPPAHAIFILATTEPHKIPPTIISRA	
B. sub	PSAVTYKVYIIDEVHMLSIGAFNALLKTLLEPPEHCIFILATTEPHKIPLTIISRC	
E. coli	PARGRFKVYLIDEVHMLSRHSFNALLKTLLEPPEHVKFLLATDPQKLPVTILSRC	
	* * * * *	***** * * * * *

FIGURE 10

10048071 102302

S. aureus	ALNIANKLERMKIYLA VGIFSLFMGADQLTTRMICSSGNVDSNRLRTGTMTTEEDWSRFTI
B. sub	ALNIAQNVA-TKTDFSVAFSLFMGAELVMRMLCAEGNINAQNLR TGNL TEEDWGKLTM
E. coli	AMNLVENAA-MLQDKPVLIFSLEMPSEQIMMRSLASLSRVDQTKIR TGQLDDEDWARISG
Sal. typ	AMNLCENAA-MLQDKPVLIFSLEMPGEQIMMRMLASLSRVDQTRIR TGQLDDEDWARISG
	* ** * * * * * * * * * * * * * * * * * * * * * * * * * * * *
S. aureus	AVGKLS-RTKIFIDDPGIPINDLR SKCRRLKQEHG-LYVIVIDYLQ LIPGVGSRASDNR
B. sub	AMGSL S-NSGIYIDDPGIRVSEIRAKCRRLKQESG-LGMILIDYLQ LIQSGG-RSKDNR
E. coli	TMGILLEKRNIIYIDDSGLTPTEVRSRARRIAREHGGIGLIMIDYLQ LMRVPA--LSDNR
Sal. typ	TMGILLEKRNMYIDDSGLTPTEVRSRARRIFREHGGLSLIMIDYLQ LMRVPS--LSDNR
	.. * * * * * * * * * * * * * * * * * * * * * * * * * * * *
S. aureus	QQEVSEISRTLKALARELECPVIADSQLSPALPPRRATRPDLPRH-----
B. sub	QQEVSEISRELKSIARELQVPVIALSQLSRGVEQRQDKRPMSDIRESGSIEQDADIVAF
E. coli	TLEIAEISRSLSKALAKELNVPVWALSQ LNRSLRQADKRPVNSDLRESGSIEQDADLIMF
Sal. typ	TLEIAEISRSLSKALAKELQVPVWALSQ LNRSLRQADKRPVNSDLRESGSIEQDADLIMF
	* ** * * * * * * * * * * * * * * * * * * * * * * * * * * * *

FIGURE 11

S. aureus α -L/ β : + + +
S. aureus $\tau\delta\delta'$ (ng): - 30 90

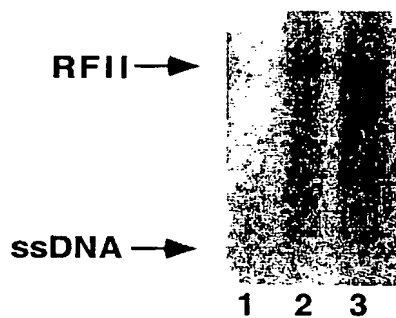


FIGURE 13

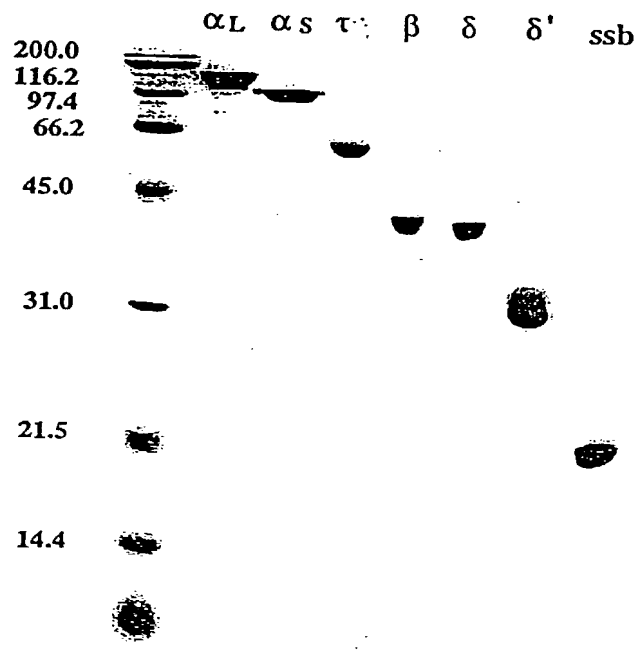
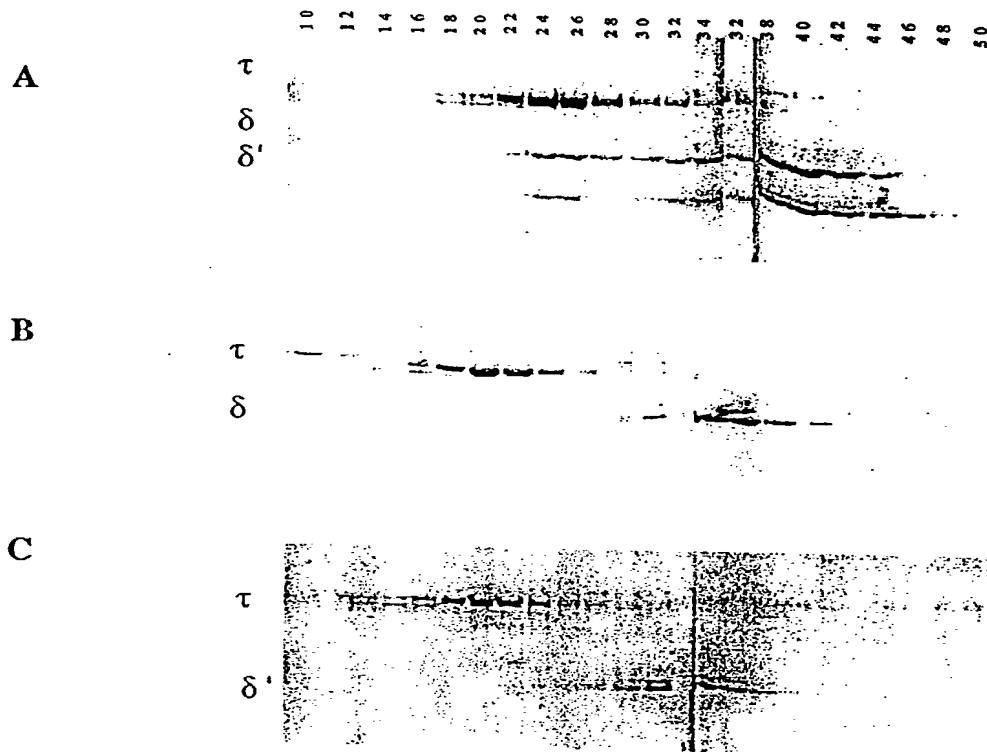


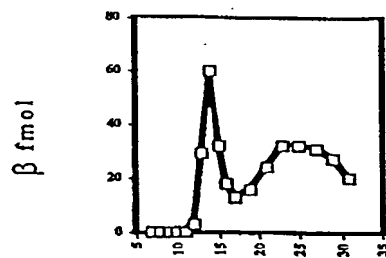
FIGURE 14

Superose 6



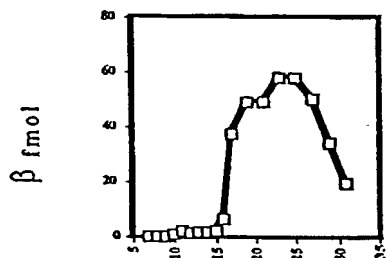
FIGURES 15A-C

A



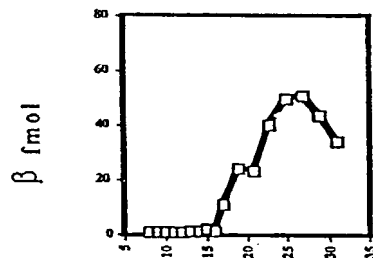
$\tau\delta\delta' + \beta$

B



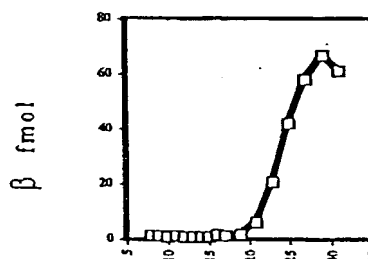
β alone

C



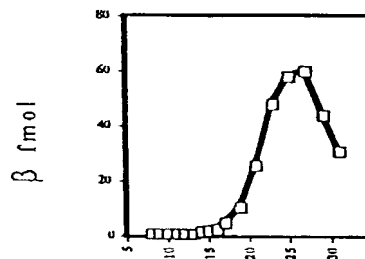
$\delta\delta' + \beta$

D



$\tau\delta + \beta$

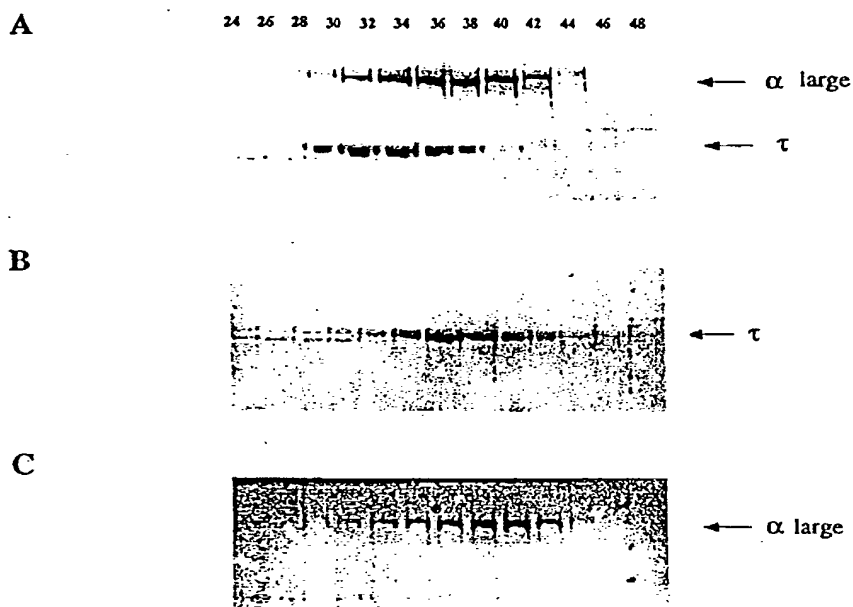
E



$\tau\delta' + \beta$

fraction

FIGURES 16A-E



FIGURES 17A-C

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18/29

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Superose 6

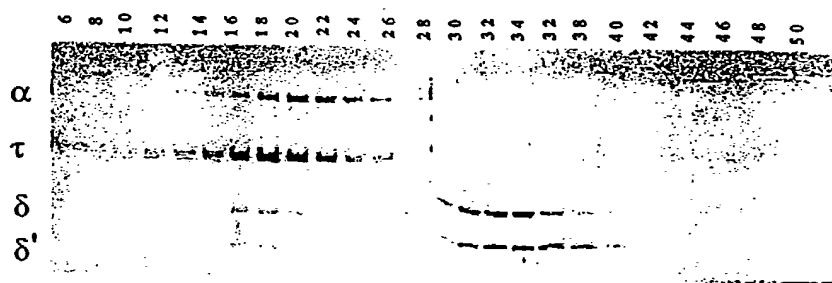


FIGURE 18

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PCT/US00/20666

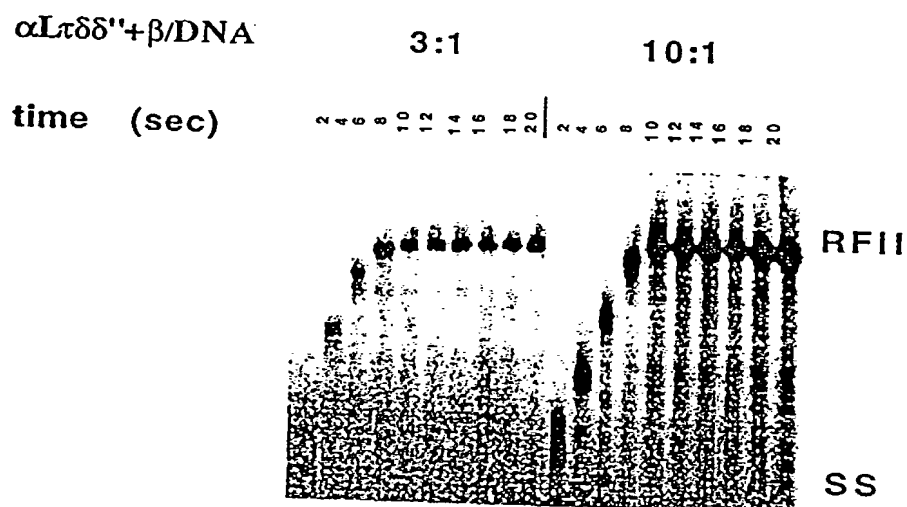


FIGURE 19

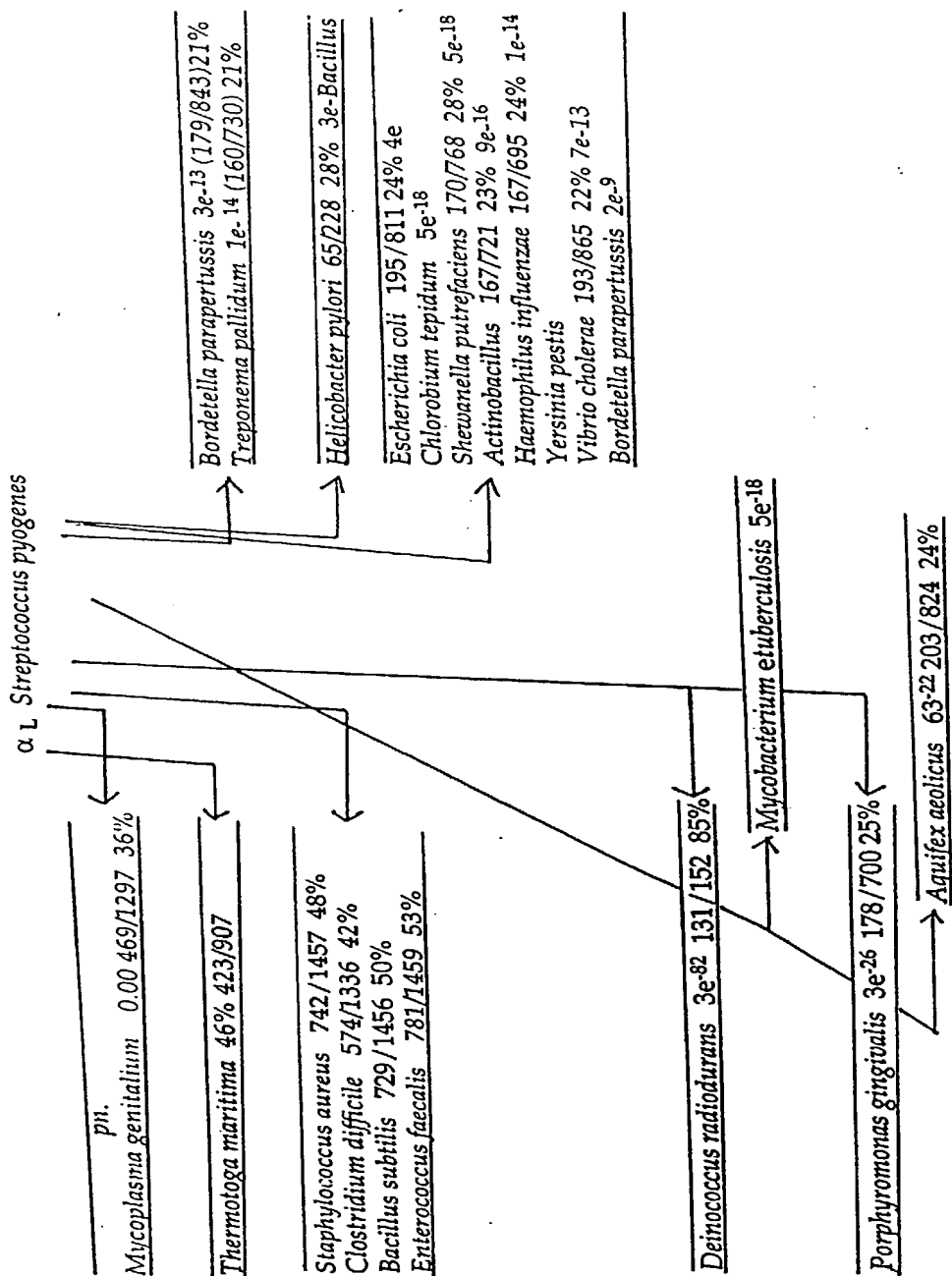
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FIGURE 20A

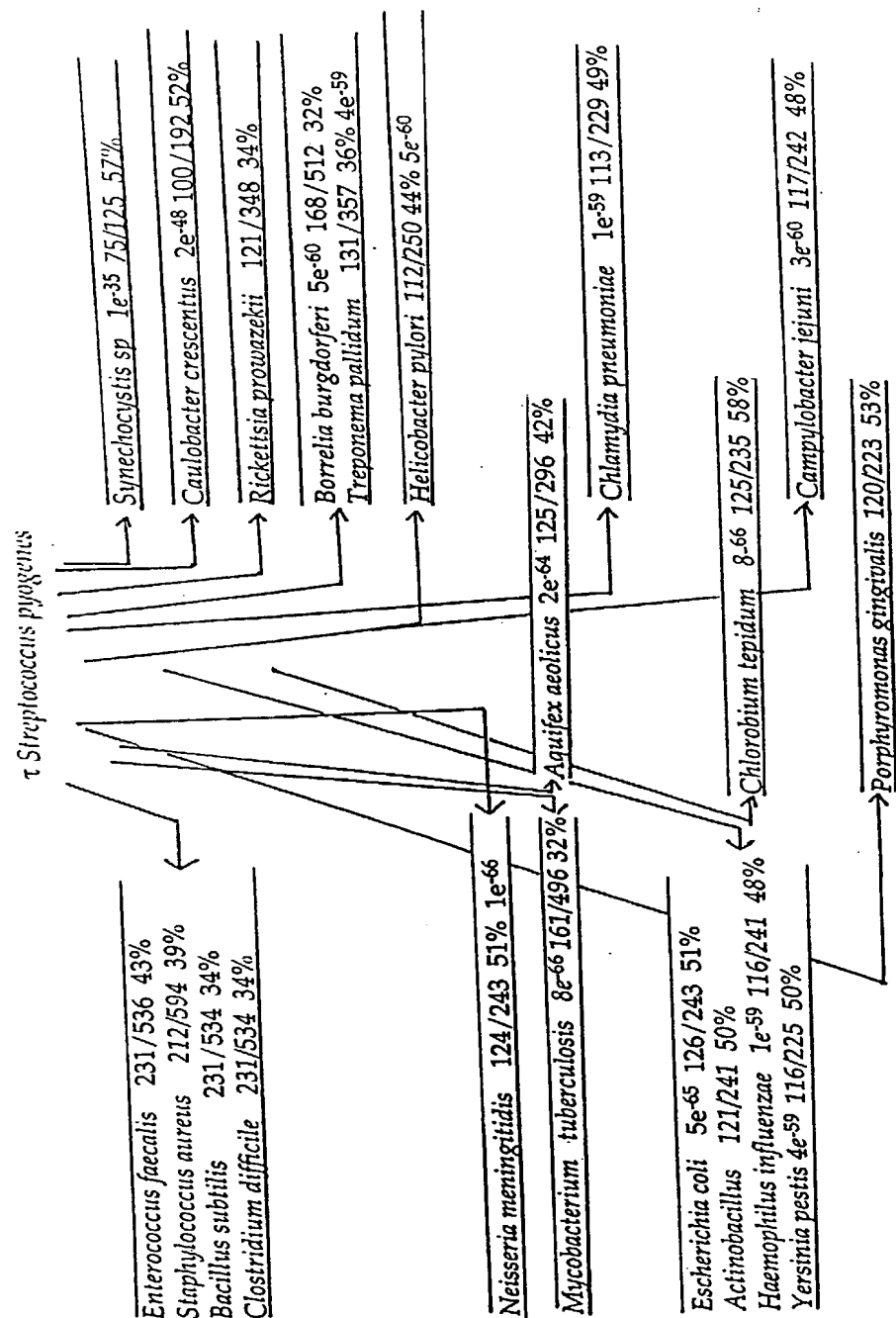


FIGURE 20B

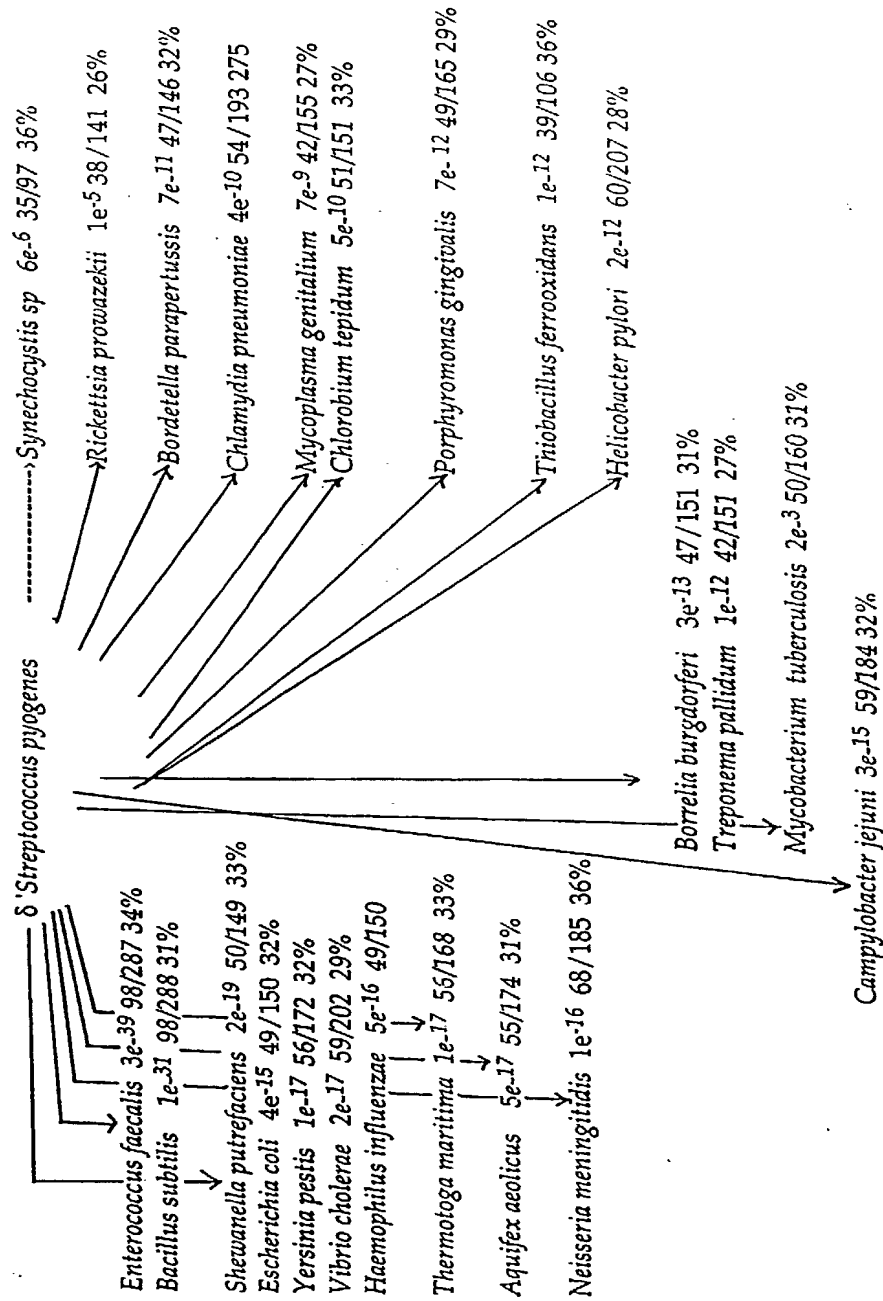


FIGURE 20C



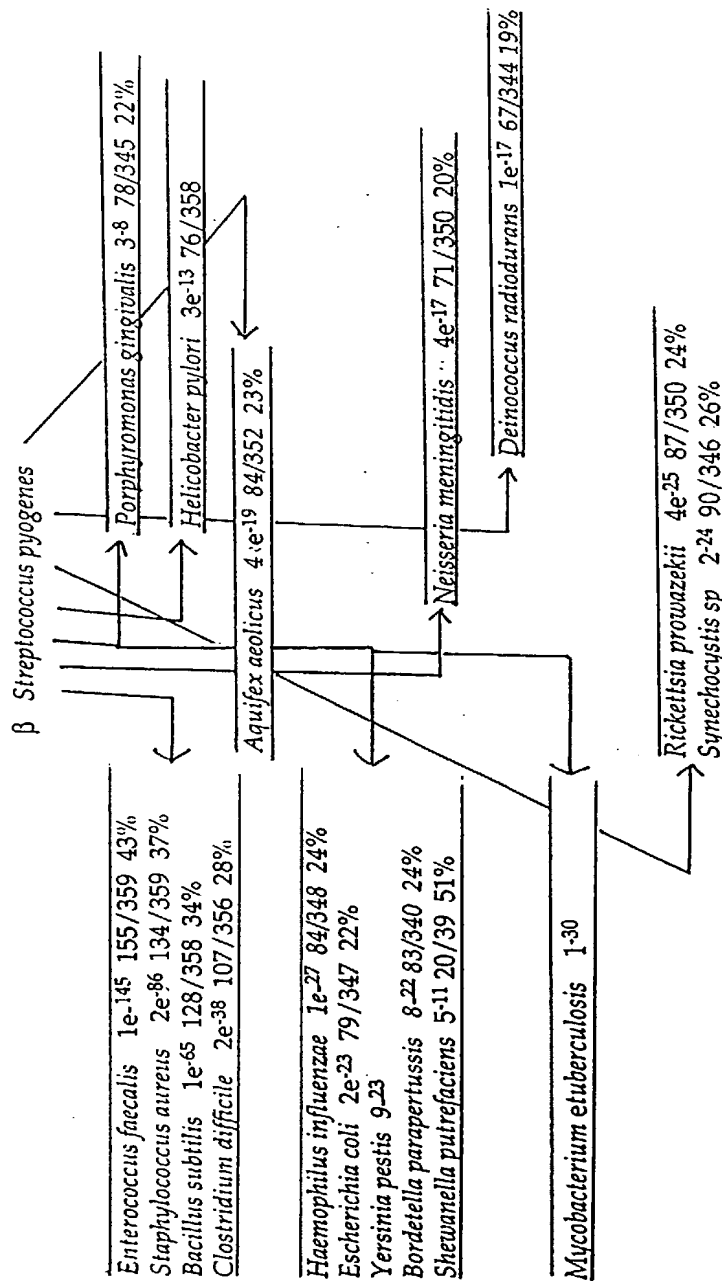


FIGURE 20E

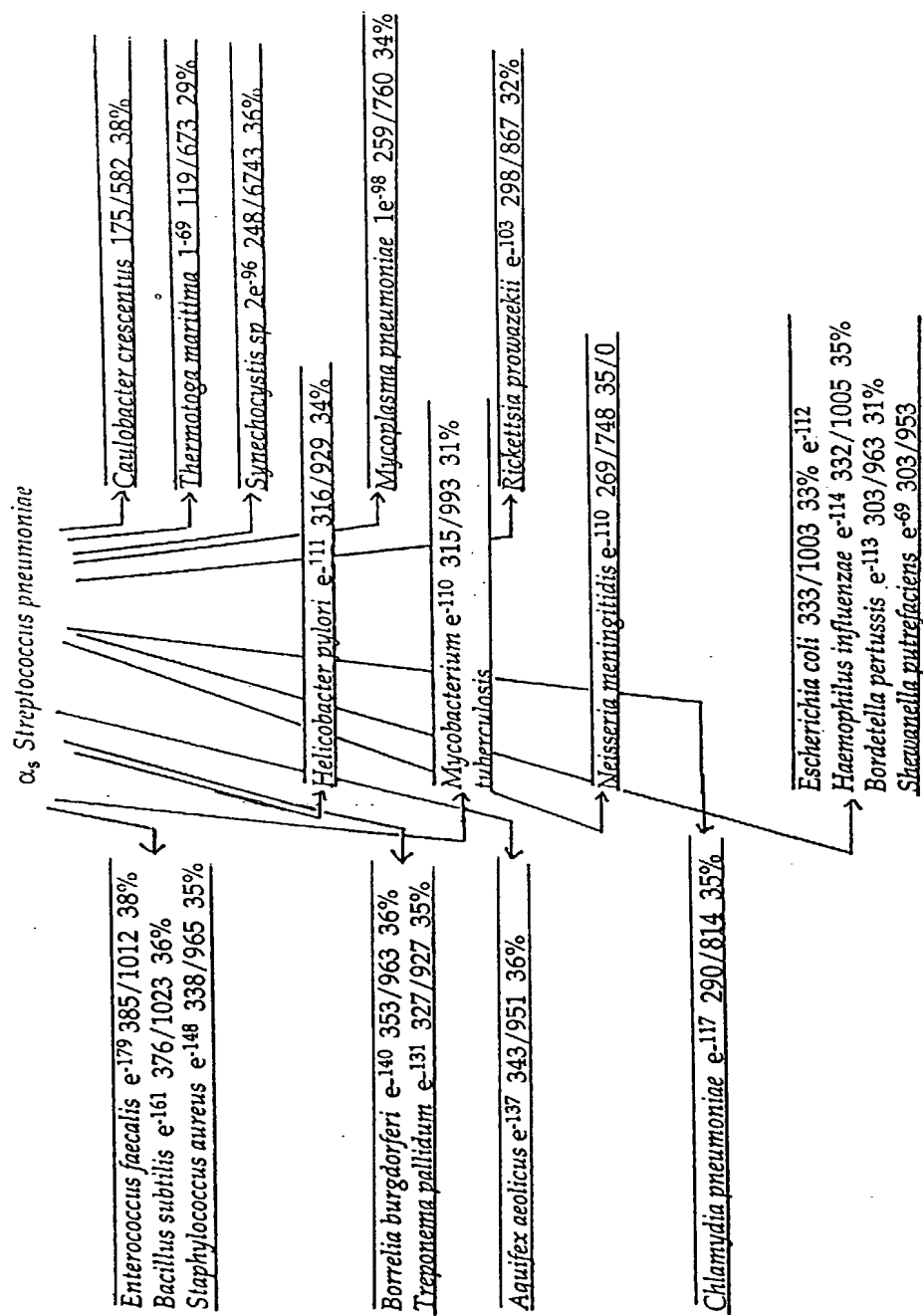


FIGURE 20F

10048071

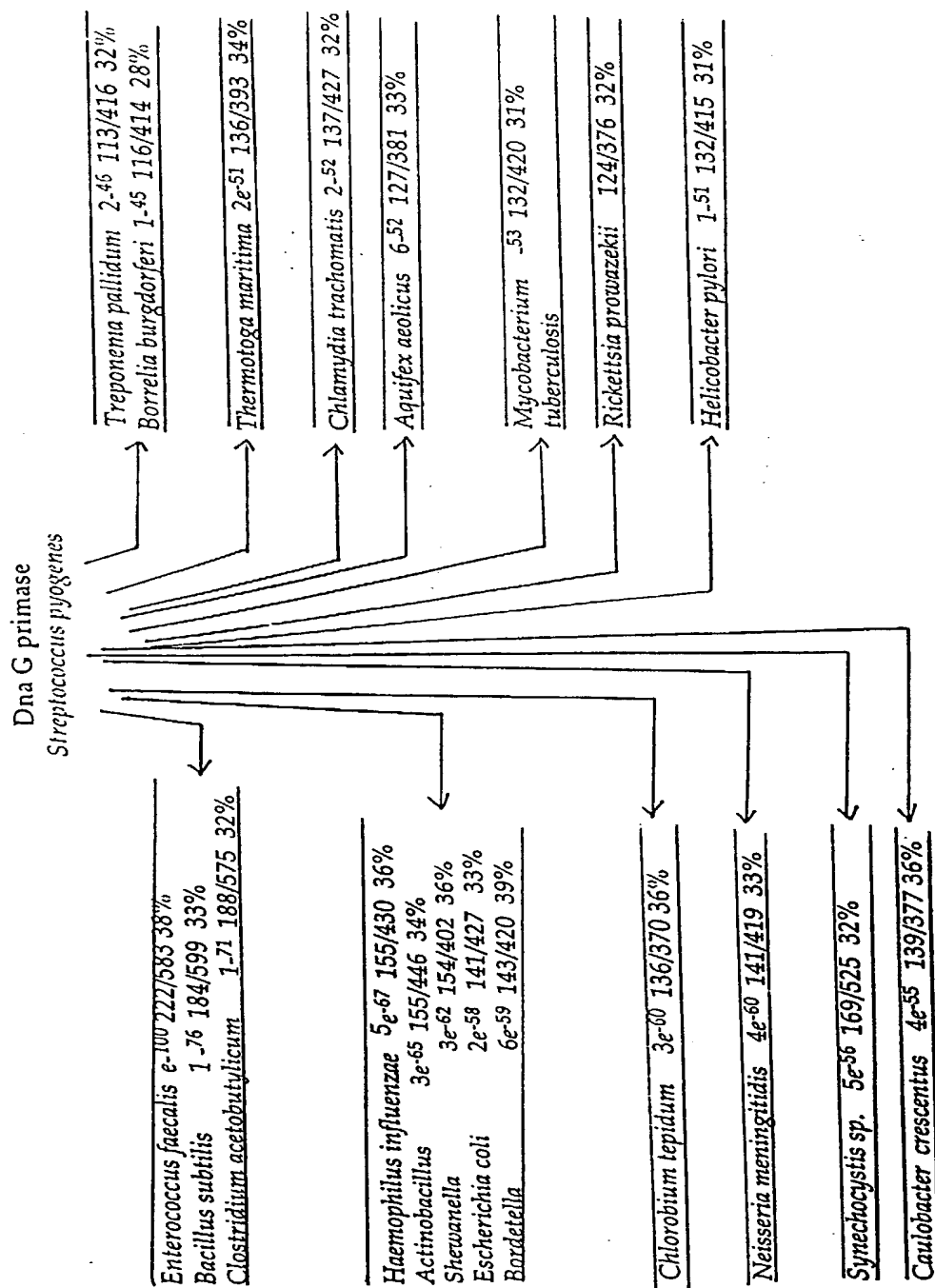


FIGURE 20G

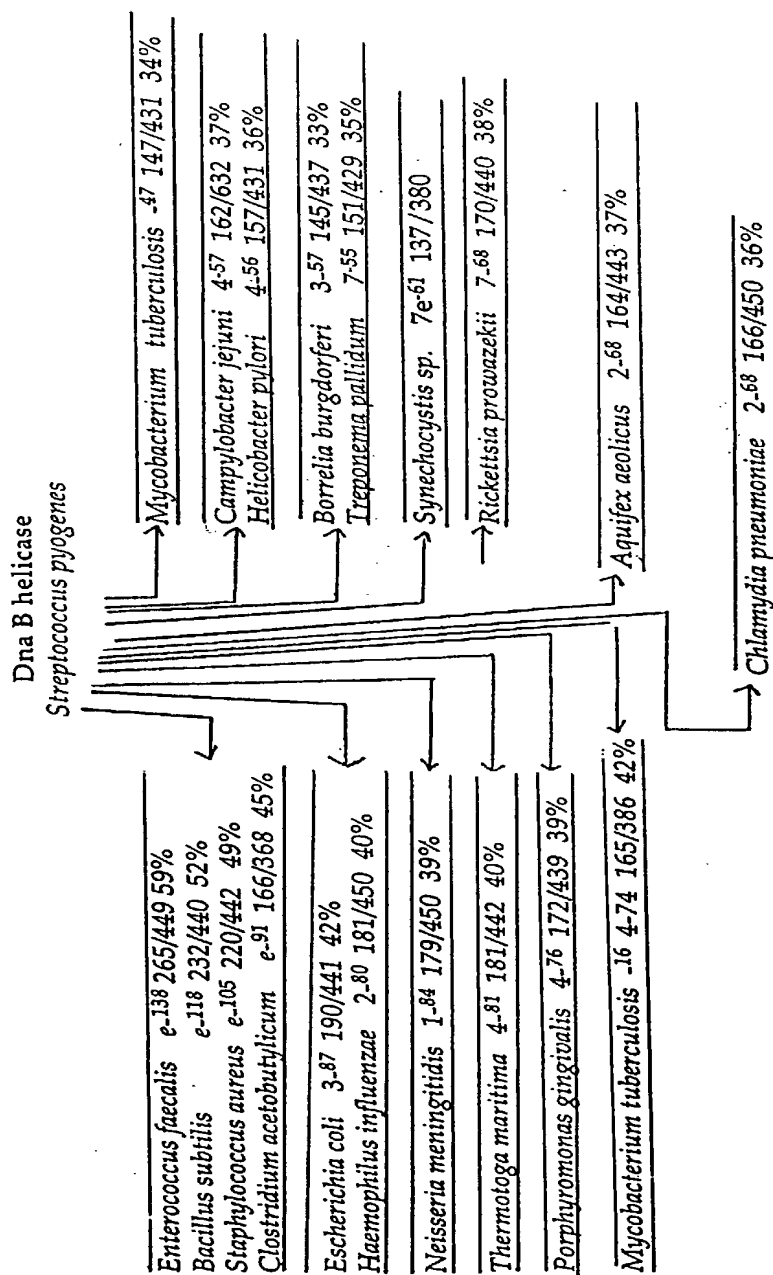


FIGURE 20H

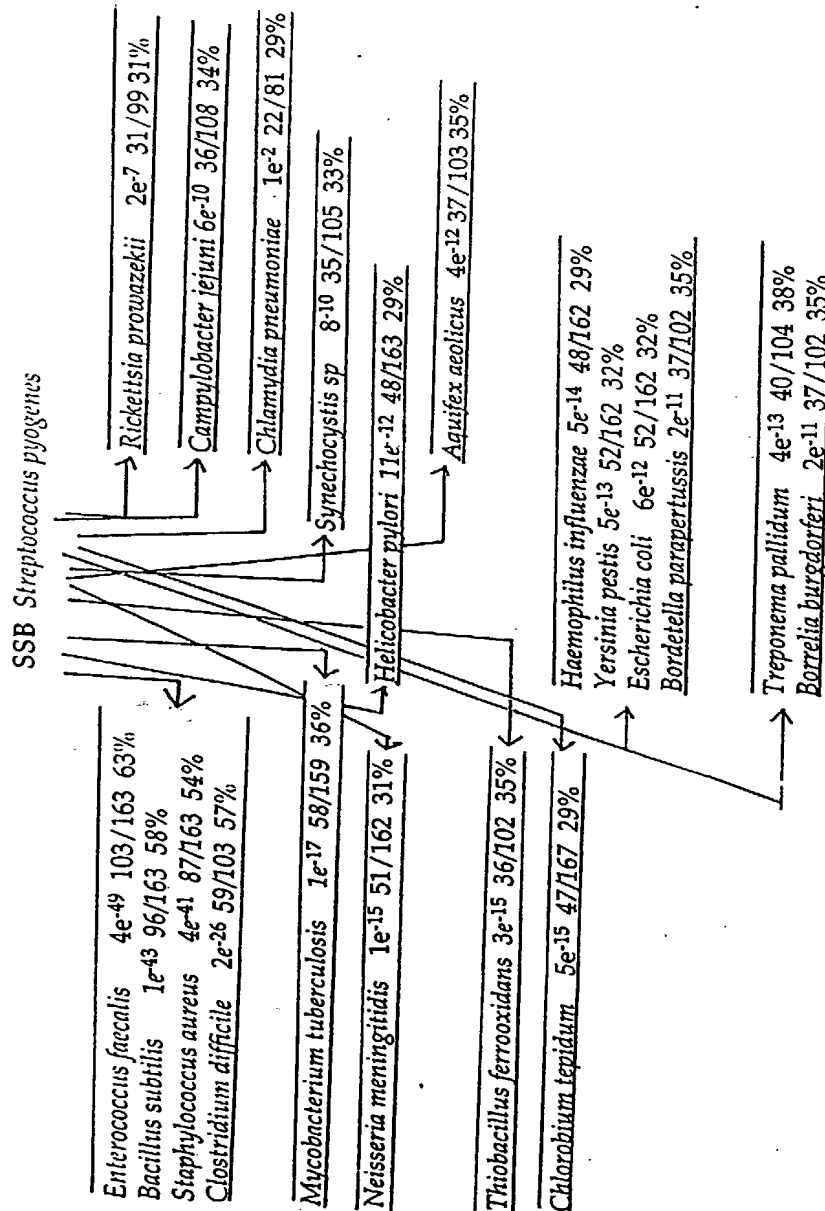
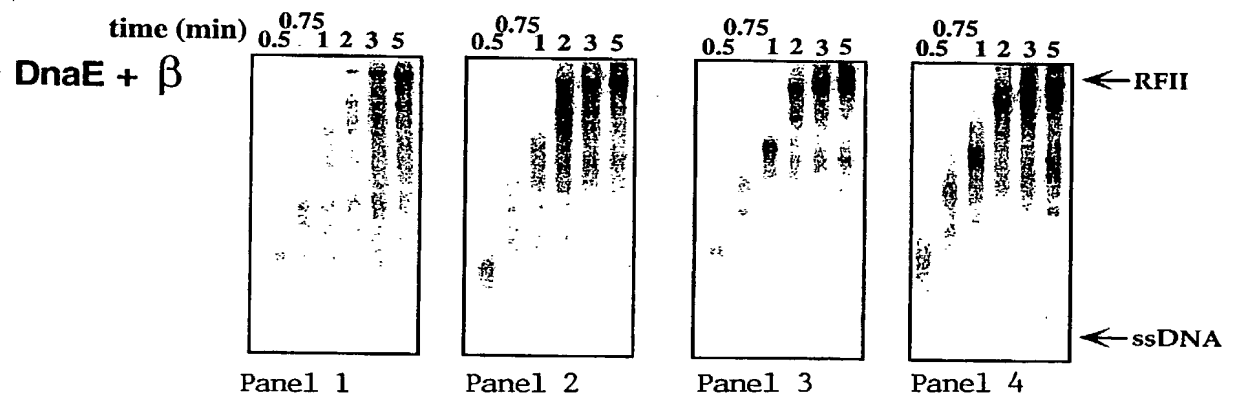
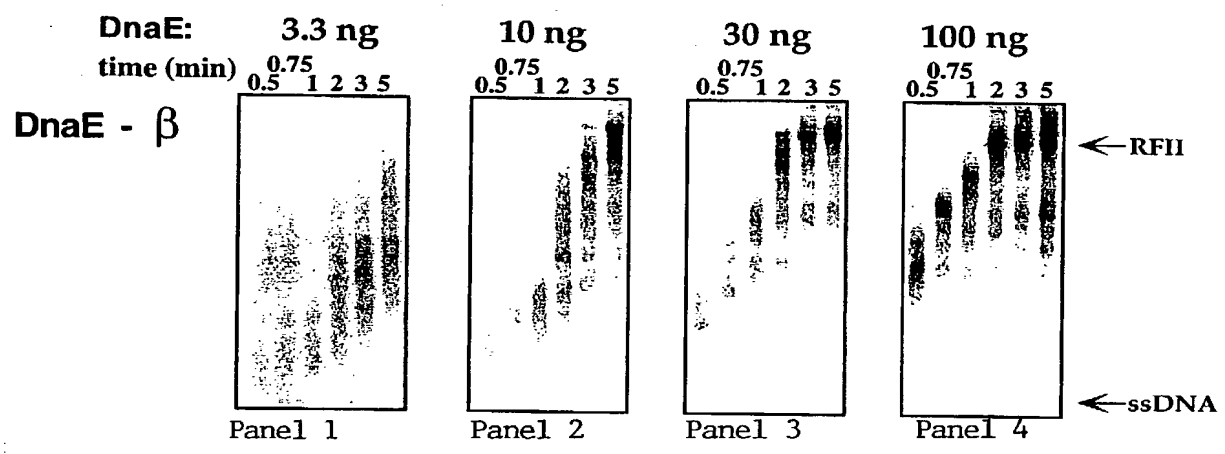
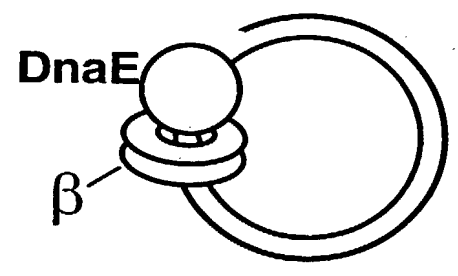
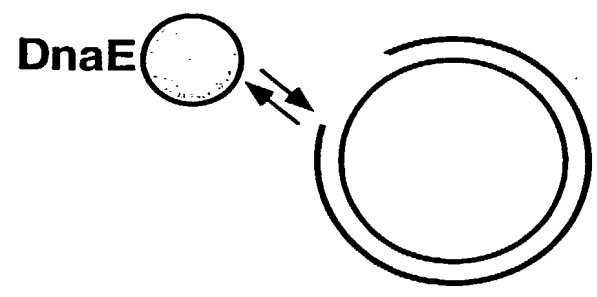
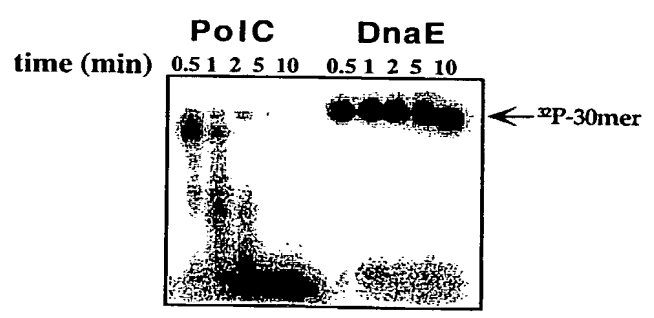


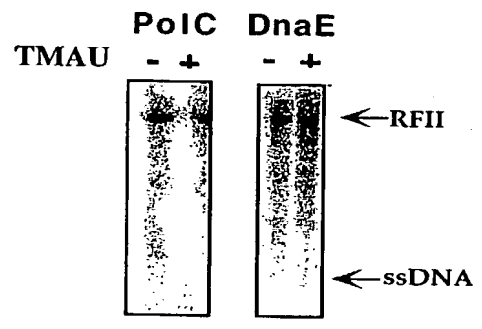
FIGURE 20I



Exonuclease assay



TMAU inhibition assay



FIGURES 21A-F

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SEQUENCE LISTING

<110> The Rockefeller University

<120> DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND
THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS

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PCT/US00/20666

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<212> PRT

<213> Staphylococcus aureus

<400> 2

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Asp Ala Leu Ala Ile Thr Asp Thr Asn Val Leu Tyr Gly Phe Pro Lys
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Phe Tyr Asp Ala Cys Ile Ala Asn Asn Ile Lys Pro Ile Phe Gly Met
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Thr Ile Tyr Val Thr Asn Gly Leu Asn Thr Val Glu Thr Val Val Leu
 65 70 75 80

Ala Lys Asn Asn Asp Gly Leu Lys Asp Leu Tyr Gln Leu Ser Ser Glu
 85 90 95

Ile Lys Met Asn Ala Leu Glu His Val Ser Phe Glu Leu Leu Lys Arg
 100 105 110

Phe Ser Asn Asn Met Ile Ile Ile Phe Lys Lys Val Gly Asp Gln His
 115 120 125

Arg Asp Ile Val Gln Val Phe Glu Thr His Asn Asp Thr Tyr Met Asp
 130 135 140

His Leu Ser Ile Ser Ile Gln Gly Arg Lys His Val Trp Ile Gln Asn
 145 150 155 160

Val Cys Tyr Gln Thr Arg Gln Asp Ala Asp Thr Ile Ser Ala Leu Ala
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Ala Ile Arg Asp Asn Thr Lys Leu Asp Leu Ile His Asp Gln Glu Asp
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Phe Gly Ala His Phe Leu Thr Glu Lys Glu Ile Asn Gln Leu Asp Ile
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Asn Gln Glu Tyr Leu Thr Gln Val Asp Val Ile Ala Gln Lys Cys Asp
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Ala Glu Leu Lys Tyr His Gln Ser Leu Leu Pro Gln Tyr Glu Thr Pro
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Asn Asp Glu Ser Ala Lys Lys Tyr Leu Trp Arg Val Leu Val Thr Gln
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Leu Lys Lys Leu Glu Leu Asn Tyr Asp Val Tyr Leu Glu Arg Leu Lys
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Tyr Glu Tyr Lys Val Ile Thr Asn Met Gly Phe Glu Asp Tyr Phe Leu
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Ile Val Ser Asp Leu Ile His Tyr Ala Lys Thr Asn Asp Val Met Val
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Gly Pro Gly Arg Gly Ser Ser Ala Gly Ser Leu Val Ser Tyr Leu Leu
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Gly Ile Thr Thr Ile Asp Pro Ile Lys Phe Asn Leu Leu Phe Glu Arg
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Glu Asp Thr Arg Arg Glu Arg Val Ile Gln Tyr Val Gln Glu Lys Tyr
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Asn His Arg His Glu Arg Trp Phe Ser Ile Cys Lys Lys Leu Glu Gly
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Pro Phe Asp Asp Gln Lys Val Phe Glu Leu Leu Ser Gln Gly Asp Thr
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Lys Leu Lys Pro Glu His Phe Glu Asp Ile Val Ala Val Thr Ser Leu
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Tyr Arg Pro Gly Pro Met Glu Glu Ile Pro Thr Tyr Ile Thr Arg Arg
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His Asp Pro Ser Lys Val Gln Tyr Leu His Pro His Leu Glu Pro Ile
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Leu Lys Asn Thr Tyr Gly Val Ile Ile Tyr Gln Glu Gln Ile Met Gln
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Ile Ala Ser Thr Phe Ala Asn Phe Ser Tyr Gly Glu Ala Asp Ile Leu
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Arg Arg Ala Met Ser Lys Lys Asn Arg Ala Val Leu Glu Ser Glu Arg
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Gln His Phe Ile Glu Gly Ala Lys Gln Asn Gly Tyr His Glu Asp Ile
 660 665 670

Ser Lys Gln Ile Phe Asp Leu Ile Leu Lys Phe Ala Asp Tyr Gly Phe
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Pro Arg Ala His Ala Val Ser Tyr Ser Lys Ile Ala Tyr Ile Met Ser
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Phe Leu Lys Val His Tyr Pro Asn Tyr Phe Tyr Ala Asn Ile Leu Ser
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Asn Val Ile Gly Ser Glu Lys Lys Thr Ala Gln Met Ile Glu Glu Ala
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Lys Lys Gln Gly Ile Thr Ile Leu Pro Pro Asn Ile Asn Glu Ser His
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Trp Phe Tyr Lys Pro Ser Gln Glu Gly Ile Tyr Leu Ser Ile Gly Thr
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Ile Lys Gly Val Gly Tyr Gln Ser Val Lys Val Ile Val Asp Glu Arg
 770 775 780

Tyr Gln Asn Gly Lys Phe Lys Asp Phe Phe Asp Phe Ala Arg Arg Ile
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Pro Lys Arg Val Lys Thr Arg Lys Leu Leu Glu Ala Leu Ile Leu Val
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Ile Asp Gln Val Leu Asp Gly Asp Leu Asn Ile Glu Gln Asp Gly Phe
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Leu Phe Asp Ile Leu Thr Pro Lys Gln Met Tyr Glu Asp Lys Glu Glu
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Leu Pro Asp Ala Leu Ile Ser Gln Tyr Glu Lys Glu Tyr Leu Gly Phe
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Tyr Val Ser Gln His Pro Val Asp Lys Lys Phe Val Ala Lys Gln Tyr
 885 890 895

Leu Thr Ile Phe Lys Leu Ser Asn Ala Gln Asn Tyr Lys Pro Ile Leu
 900 905 910

Val Gln Phe Asp Lys Val Lys Gln Ile Arg Thr Lys Asn Gly Gln Asn
 915 920 925

Met Ala Phe Val Thr Leu Asn Asp Gly Ile Glu Thr Leu Asp Gly Val
 930 935 940

Ile Phe Pro Asn Gln Phe Lys Lys Tyr Glu Glu Leu Leu Ser His Asn
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Asp Leu Phe Ile Val Ser Gly Lys Phe Asp His Arg Lys Gln Gln Arg
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Gln Leu Ile Ile Asn Glu Ile Gln Thr Leu Ala Thr Phe Glu Glu Gln
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Lys Leu Ala Phe Ala Lys Gln Ile Ile Ile Arg Asn Lys Ser Gln Ile
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 1010 1015 1020

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<212> DNA

<213> *Staphylococcus aureus*

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275	280	285
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Arg Ala Leu Met Asn Leu Glu Leu Asp Met Leu Tyr Gln Met Ile Asp		
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Leu Ile Asn Asp Thr Leu Val Ser Ile Arg Phe Ser Val Asn Gln Asn		
325	330	335
Val His Phe Glu Val Leu Leu Val Lys Leu Ala Glu Gln Ile Lys Gly		
340	345	350
Gln Pro Gln Val Ile Ala Asn Val Ala Glu Pro Ala Gln Ile Ala Ser		
355	360	365
Ser Pro Asn Thr Asp Val Leu Leu Gln Arg Met Glu Gln Leu Glu Gln		
370	375	380
Glu Leu Lys Thr Leu Lys Ala Gln Gly Val Ser Val Ala Pro Thr Gln		
385	390	395
Lys Ser Ser Lys Lys Pro Ala Arg Gly Ile Gln Lys Ser Lys Asn Ala		
405	410	415
Phe Ser Met Gln Gln Ile Ala Lys Val Leu Asp Lys Ala Asn Lys Ala		
420	425	430
Asp Ile Lys Leu Leu Lys Asp His Trp Gln Glu Val Ile Asp His Ala		
435	440	445
Gln Asn Asn Asp Lys Lys Ser Leu Val Ser Leu Leu Gln Asn Ser Glu		
450	455	460
Pro Val Ala Ala Ser Glu Asp His Val Leu Val Lys Phe Glu Glu Glu		
465	470	475
Ile His Cys Glu Ile Val Asn Lys Asp Asp Glu Lys Arg Ser Ser Ile		
485	490	495
Glu Ser Val Val Cys Asn Ile Val Asn Lys Asn Val Lys Val Val Gly		
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Val Pro Ser Asp Gln Trp Gln Arg Val Arg Thr Glu Tyr Leu Gln Asn		
515	520	525
Arg Lys Asn Glu Gly Asp Asp Met Pro Lys Gln Gln Ala Gln Gln Thr		

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530

535

540

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Val Ile Asp Glu Glu Glx
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<211> 1398

<212> DNA

<213> *Staphylococcus aureus*

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<211> 466

<212> PRT

<213> *Staphylococcus aureus*

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Thr Thr Gln Glu Val Leu Leu Pro Glu Ser Phe Tyr Arg Gly Ala His	35	40	45
Gln His Ile Phe Arg Ala Met Met His Leu Asn Glu Asp Asn Lys Glu	50	55	60
Ile Asp Val Val Thr Leu Met Asp Gln Leu Ser Thr Glu Gly Thr Leu	65	70	75
Asn Glu Ala Gly Gly Pro Gln Tyr Leu Ala Glu Leu Ser Thr Asn Val	85	90	95
Pro Thr Thr Arg Asn Val Gln Tyr Tyr Thr Asp Ile Val Ser Lys His	100	105	110
Ala Leu Lys Arg Arg Leu Ile Gln Thr Ala Asp Ser Ile Ala Asn Asp	115	120	125
Gly Tyr Asn Asp Glu Leu Glu Leu Asp Ala Ile Leu Ser Asp Ala Glu	130	135	140
Arg Arg Ile Leu Glu Leu Ser Ser Ser Arg Glu Ser Asp Gly Phe Lys	145	150	155
Asp Ile Arg Asp Val Leu Gly Gln Val Tyr Glu Thr Ala Glu Glu Leu	165	170	175
Asp Gln Asn Ser Gly Gln Thr Pro Gly Ile Pro Thr Gly Tyr Arg Asp	180	185	190
Leu Asp Gln Met Thr Ala Gly Phe Asn Arg Asn Asp Leu Ile Ile Leu	195	200	205
Ala Ala Arg Pro Ser Val Gly Lys Thr Ala Phe Ala Leu Asn Ile Ala	210	215	220
Gln Lys Val Ala Thr His Glu Asp Met Tyr Thr Val Gly Ile Phe Ser	225	230	235
Leu Glu Met Gly Ala Asp Gln Leu Ala Thr Arg Met Ile Cys Ser Ser	245	250	255
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290	295	300
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305	310	315
Asp Tyr Leu Gln Leu Ile Gln Gly Ser Gly Ser Arg Ala Ser Asp Asn		
325	330	335
Arg Gln Gln Glu Val Ser Glu Ile Ser Arg Thr Leu Lys Ala Leu Ala		
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Arg Glu Leu Lys Cys Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly		
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Val Glu Gln Arg Gln Asp Lys Arg Pro Met Met Ser Asp Ile Arg Glu		
370	375	380
Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg		
385	390	395
Asp Asp Tyr Tyr Asn Arg Gly Gly Asp Glu Asp Asp Asp Asp Asp Gly		
405	410	415
Gly Phe Glu Pro Gln Thr Asn Asp Glu Asn Gly Glu Ile Glu Ile Ile		
420	425	430
Ile Ala Lys Gln Arg Asn Gly Pro Thr Gly Thr Val Lys Leu His Phe		
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Met Met		
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<210> 7

<211> 4308

<212> DNA

<213> Staphylococcus aureus

<400> 7

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<211> 1435

<212> PRT

<213> *Staphylococcus aureus*

<400> 8

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Ile Asp Val Ser Asn Lys Asn Arg Thr Trp Glu Phe His Ile Thr Leu
      35              40              45

Pro Gln Phe Leu Ala His Glu Asp Tyr Leu Leu Phe Ile Asn Ala Ile
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Glu Gln Glu Phe Lys Asp Ile Ala Asn Val Thr Cys Arg Phe Thr Val
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Thr Asn Gly Thr Asn Gln Asp Glu His Ala Ile Lys Tyr Phe Gly His

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Thr Asp His Asn Val Val Gln Ala Phe Pro Asp Ala His Ala Ala Ala		
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Glu Lys His Gly Ile Lys Met Ile Tyr Gly Met Glu Gly Met Leu Val		
385	390	395
Asp Asp Gly Val Pro Ile Ala Tyr Lys Pro Gln Asp Val Val Leu Lys		
	405	410
		415
Asp Ala Thr Tyr Val Val Phe Asp Val Glu Thr Thr Gly Leu Ser Asn		
	420	425
		430
Gln Tyr Asp Lys Ile Ile Glu Leu Ala Ala Val Lys Val His Asn Gly		
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		445
Glu Ile Ile Asp Lys Phe Glu Arg Phe Ser Asn Pro His Glu Arg Leu		
	450	455
		460
Ser Glu Thr Ile Ile Asn Leu Thr His Ile Thr Asp Asp Met Leu Val		
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		475
Asp Ala Pro Glu Ile Glu Glu Val Leu Thr Glu Phe Lys Glu Trp Val		
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		495
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		510
Ile Asp Thr Gly Tyr Glu Arg Leu Gly Phe Gly Pro Ser Thr Asn Gly		
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		525
Val Ile Asp Thr Leu Glu Leu Ser Arg Thr Ile Asn Thr Glu Tyr Gly		
	530	535
		540
Lys His Gly Leu Asn Phe Leu Ala Lys Lys Tyr Gly Val Glu Leu Thr		
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		555
Gln His His Arg Ala Ile Tyr Asp Thr Glu Ala Thr Ala Tyr Ile Phe		
	565	570
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Ile Lys Met Val Gln Gln Met Lys Glu Leu Gly Val Leu Asn His Asn		
	580	585
		590
Glu Ile Asn Lys Lys Leu Ser Asn Glu Asp Ala Tyr Lys Arg Ala Arg		

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595	600	605
Pro Ser His Val Thr Leu Ile Val Gln Asn Gln Gln Gly Leu Lys Asn		
610	615	620
Leu Phe Lys Ile Val Ser Ala Ser Leu Val Lys Tyr Phe Tyr Arg Thr		
625	630	635 640
Pro Arg Ile Pro Arg Ser Leu Leu Asp Glu Tyr Arg Glu Gly Leu Leu		
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Val Gly Thr Ala Cys Asp Glu Gly Glu Leu Phe Thr Ala Val Met Gln		
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Lys Asp Gln Ser Gln Val Glu Lys Ile Ala Lys Tyr Tyr Asp Phe Ile		
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Glu Ile Gln Pro Pro Ala Leu Tyr Gln Asp Leu Ile Asp Arg Glu Leu		
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Ile Arg Asp Thr Glu Thr Leu His Glu Ile Tyr Gln Arg Leu Ile His		
705	710	715 720
Ala Gly Asp Thr Ala Gly Ile Pro Val Ile Ala Thr Gly Asn Ala His		
	725	730 735
Tyr Leu Phe Glu His Asp Gly Ile Ala Arg Lys Ile Leu Ile Ala Ser		
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Gln Pro Gly Asn Pro Leu Asn Arg Ser Thr Leu Pro Glu Ala His Phe		
	755	760 765
Arg Thr Thr Asp Glu Met Leu Asn Glu Phe His Phe Leu Gly Glu Glu		
	770	775 780
Lys Ala His Glu Ile Val Val Lys Asn Thr Asn Glu Leu Ala Asp Arg		
785	790	795 800
Ile Glu Arg Val Val Pro Ile Lys Asp Glu Leu Tyr Thr Pro Arg Met		
	805	810 815
Glu Gly Ala Asn Glu Glu Ile Arg Glu Leu Ser Tyr Ala Asn Ala Arg		
	820	825 830
Lys Leu Tyr Gly Glu Asp Leu Pro Gln Ile Val Ile Asp Arg Leu Glu		
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Lys Glu Leu Lys Ser Ile Ile Gly Asn Gly Phe Ala Val Ile Tyr Leu		

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Gly	Ser	Arg	Gly	Ser	Val	Gly	Ser	Ser	Phe	Val	Ala	Thr	Met	Thr	Glu	
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Ile	Thr	Glu	Val	Asn	Pro	Leu	Pro	Pro	His	Tyr	Ile	Cys	Pro	Asn	Cys	
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Lys	Thr	Ser	Glu	Phe	Phe	Asn	Asp	Gly	Ser	Val	Gly	Ser	Gly	Phe	Asp	
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Leu	Pro	Asp	Lys	Thr	Cys	Glu	Thr	Cys	Gly	Ala	Pro	Leu	Ile	Lys	Glu	
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His	Asn	Tyr	Thr	Lys	Val	Leu	Phe	Gly	Glu	Asp	Lys	Val	Phe	Arg	Ala	
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Gln Ile Phe Ser Thr Pro Glu Ser Leu Gly Val Thr Glu Asp Glu Ile			
1125	1130	1135	
Leu Cys Lys Thr Gly Thr Phe Gly Val Pro Asn Ser Asp Arg Ile Arg			
1140	1145	1150	
Arg Gln Met Leu Glu Asp Thr Lys Pro Thr Thr Phe Ser Glu Leu Val			
1155	1160	1165	
Gln Ile Ser Gly Leu Ser His Gly Thr Asp Val Trp Leu Gly Asn Ala			
1170	1175	1180	
Gln Glu Leu Ile Lys Thr Gly Ile Cys Asp Leu Ser Ser Val Ile Gly			
1185	1190	1195	1200
Cys Arg Asp Asp Ile Met Val Tyr Leu Met Tyr Ala Gly Leu Glu Pro			
1205	1210	1215	
Ser Met Ala Phe Lys Ile Met Glu Ser Val Arg Lys Gly Lys Gly Leu			
1220	1225	1230	
Thr Glu Glu Met Ile Glu Thr Met Lys Glu Asn Glu Val Pro Asp Trp			
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Tyr Leu Asp Ser Cys Leu Lys Ile Lys Tyr Ile Phe Pro Lys Ala His			
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Ala Ala Ala Tyr Val Leu Met Ala Val Arg Ile Ala Tyr Phe Lys Val			
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His His Pro Leu Tyr Tyr Tyr Ala Ser Tyr Phe Thr Ile Arg Ala Ser			
1285	1290	1295	
Asp Phe Asp Leu Ile Thr Met Ile Lys Asp Lys Thr Ser Ile Arg Asn			
1300	1305	1310	
Thr Val Lys Asp Met Tyr Ser Arg Tyr Met Asp Leu Gly Lys Lys Glu			
1315	1320	1325	
Lys Asp Val Leu Thr Val Leu Glu Ile Met Asn Glu Met Ala His Arg			
1330	1335	1340	
Gly Tyr Arg Met Gln Pro Ile Ser Leu Glu Lys Ser Gln Ala Phe Glu			
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Phe Ile Ile Glu Gly Asp Thr Leu Ile Pro Pro Phe Ile Ser Val Pro			

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Gly Pro Phe Leu Ser Lys Glu Asp Leu Asn Lys Lys Ala Gly Leu Tyr			
1395	1400	1405	
Gln Lys Ile Ile Glu Tyr Leu Asp Glu Leu Gly Ser Leu Pro Asn Leu			
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<211> 1134

<212> DNA

<213> Staphylococcus aureus

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<212> PRT

<213> Staphylococcus aureus

<400> 10

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 20 25 30
 Gly Ile Lys Ile Asp Ala Lys Glu His Glu Val Ile Leu Thr Gly Ser
 35 40 45
 Asp Ser Glu Ile Ser Ile Glu Ile Thr Ile Pro Lys Thr Val Asp Gly
 50 55 60
 Glu Asp Ile Val Asn Ile Ser Glu Thr Gly Ser Val Val Leu Pro Gly
 65 70 75 80
 Arg Phe Phe Val Asp Ile Ile Lys Lys Leu Pro Gly Lys Asp Val Lys
 85 90 95
 Leu Ser Thr Asn Glu Gln Phe Gln Thr Leu Ile Thr Ser Gly His Ser
 100 105 110
 Glu Phe Asn Leu Ser Gly Leu Asp Pro Asp Gln Tyr Pro Leu Leu Pro
 115 120 125
 Gln Val Ser Arg Asp Asp Ala Ile Gln Leu Ser Val Lys Val Leu Lys
 130 135 140
 Asn Val Ile Ala Gln Thr Asn Phe Ala Val Ser Thr Ser Glu Thr Arg
 145 150 155 160
 Pro Val Leu Thr Gly Val Asn Trp Leu Ile Gln Glu Asn Glu Leu Ile
 165 170 175
 Cys Thr Ala Thr Asp Ser His Arg Leu Ala Val Arg Lys Leu Gln Leu
 180 185 190
 Glu Asp Val Ser Glu Asn Lys Asn Val Ile Ile Pro Gly Lys Ala Leu
 195 200 205
 Ala Glu Leu Asn Lys Ile Met Ser Asp Asn Glu Glu Asp Ile Asp Ile
 210 215 220
 Phe Phe Ala Ser Asn Gln Val Leu Phe Lys Val Gly Asn Val Asn Phe
 225 230 235 240
 Ile Ser Arg Leu Leu Glu Gly His Tyr Pro Asp Thr Thr Arg Leu Phe
 245 250 255

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Pro Glu Asn Tyr Glu Ile Lys Leu Ser Ile Asp Asn Gly Glu Phe Tyr
 260 265 270

His Ala Ile Asp Arg Ala Ser Leu Leu Ala Arg Glu Gly Gly Asn Asn
 275 280 285

Val Ile Lys Leu Ser Thr Gly Asp Asp Val Val Glu Leu Ser Ser Thr
 290 295 300

Ser Pro Glu Ile Gly Thr Val Lys Glu Glu Val Asp Ala Asn Asp Val
 305 310 315 320

Glu Gly Gly Ser Leu Lys Ile Ser Phe Asn Ser Lys Tyr Met Met Asp
 325 330 335

Ala Leu Lys Ala Ile Asp Asn Asp Glu Val Glu Val Glu Phe Phe Gly
 340 345 350

Thr Met Lys Pro Phe Ile Leu Lys Pro Lys Gly Asp Asp Ser Val Thr
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Gln Leu Ile Leu Pro Ile Arg Thr Tyr
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<211> 930

<212> DNA

<213> Staphylococcus aureus

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<212> PRT

<213> Staphylococcus aureus

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 35 40 45

Cys Glu Thr Lys Val Ser Thr Tyr Asn His Pro Asp Phe Met Tyr Ile
 50 55 60

Ser Thr Thr Glu Asn Ala Ile Lys Lys Glu Gln Val Glu Gln Leu Val
 65 70 75 80

Arg His Met Asn Gln Leu Pro Ile Glu Ser Thr Asn Lys Val Tyr Ile
 85 90 95

Ile Glu Asp Phe Glu Asp Phe Glu Lys Leu Thr Val Gln Gly Glu Asn
 100 105 110

Ser Ile Leu Lys Phe Leu Glu Glu Pro Pro Asp Asn Thr Ile Ala Ile
 115 120 125

Leu Leu Ser Thr Lys Pro Glu Gln Ile Leu Asp Thr Ile His Ser Arg
 130 135 140

Cys Gln His Val Tyr Phe Lys Pro Ile Asp Lys Glu Lys Phe Ile Asn
 145 150 155 160

Arg Leu Val Glu Gln Asn Met Ser Lys Pro Val Ala Glu Met Ile Ser
 165 170 175

Thr Tyr Thr Thr Gln Ile Asp Asn Ala Met Ala Leu Asn Glu Glu Phe
 180 185 190

Asp Leu Leu Ala Leu Arg Lys Ser Val Ile Arg Trp Glu Leu Leu Leu
 195 200 205

Thr Asn Lys Pro Met Ala Leu Ile Gly Ile Ile Asp Leu Leu Lys Gln
 210 215 220

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Ala Lys Asn Lys Lys Leu Gln Ser Leu Thr Ile Ala Ala Val Asn Gly
 225 230 235 240

Phe Phe Glu Asp Ile Ile His Thr Lys Val Asn Val Glu Asp Lys Gln
 245 250 255

Ile Tyr Ser Asp Leu Lys Asn Asp Ile Asp Gln Tyr Ala Gln Lys Leu
 260 265 270

Ser Phe Asn Gln Leu Ile Leu Met Phe Asp Gln Leu Thr Glu Ala His
 275 280 285

Lys Lys Leu Asn Gln Asn Val Asn Pro Thr Leu Val Phe Glu Gln Ile
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Val Ile Lys Gly Val Ser
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<210> 13

<211> 744

<212> DNA

<213> Staphylococcus aureus

<400> 13

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<210> 14

<211> 248

<212> PRT

<213> Staphylococcus aureus

<400> 14

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Asp Asp Phe Asn Phe Val Lys Tyr Asn Leu Tyr Glu Thr Glu Ile Ala	35	40	45
Pro Ile Val Glu Glu Thr Leu Thr Leu Pro Phe Phe Ser Asp Lys Lys	50	55	60
Ala Ile Leu Val Lys Asn Ala Tyr Ile Phe Thr Gly Glu Lys Ala Pro	65	70	75
Lys Asp Met Ala His Asn Val Asp Gln Leu Ile Glu Phe Ile Glu Lys	85	90	95
Tyr Asp Gly Glu Asn Leu Ile Val Phe Glu Ile Tyr Gln Asn Lys Leu	100	105	110
Asp Glu Arg Lys Lys Leu Thr Lys Thr Leu Lys Lys His Ala Arg Leu	115	120	125
Lys Lys Ile Glu Gln Met Ser Glu Glu Ile Lys Trp Ile Gln Lys Lys	130	135	140
Glu Gln Ala Ile Asp Phe Val Lys Asp Leu Ile Thr Met Lys Glu Glu	145	150	155
Pro Ile Lys Leu Leu Ala Leu Thr Ser Asn Tyr Arg Leu Phe Tyr Gln	165	170	175
Cys Lys Ile Leu Ser Gln Lys Gly Tyr Ser Gly Gln Gln Ile Ala Lys	180	185	190
Thr Ile Gly Val His Pro Tyr Arg Val Lys Leu Ala Leu Gly Gln Val	195	200	205
Arg His Tyr Gln Leu Asp Glu Leu Leu Asn Ile Ile Asp Ala Cys Ala	210	215	220
Glu Thr Asp Tyr Lys Leu Lys Ser Ser Tyr Met Asp Lys Gln Leu Ile	225	230	235
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<212> DNA

<213> *Staphylococcus aureus*

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Glu Ala Val Lys Glu Leu Gly Asp Arg Val Asn Val Ala Val Asp Ile
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Glu Ala Thr Gln Ser Asn Ser Asn Val Gln Ile Ala Ser Asp Asp Leu
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Gln Met Ile Glu Met His Glu Leu Ile Gln Glu Phe Tyr Tyr Tyr Ala
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Leu Thr Lys Thr Val Glu Gly Glu Gln Ala Leu Thr Tyr Leu Gln Glu
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Arg Gly Phe Thr Asp Ala Leu Ile Lys Glu Arg Gly Ile Gly Phe Ala
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Pro Asp Ser Ser His Phe Cys His Asp Phe Leu Gln Lys Lys Gly Tyr
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Asn Phe Ser Tyr Tyr Asp Arg Phe Arg Asn Arg Ile Met Phe Pro Leu
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Lys Asn Ala Gln Gly Arg Ile Val Gly Tyr Ser Gly Arg Thr Tyr Thr
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Gly Gln Glu Pro Lys Tyr Leu Asn Ser Pro Glu Thr Pro Ile Phe Gln
 195 200 205

Lys Arg Lys Leu Leu Tyr Asn Leu Asp Lys Ala Arg Lys Ser Ile Arg
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Lys Leu Asp Glu Ile Val Leu Leu Glu Gly Phe Met Asp Val Ile Lys
 225 230 235 240

Ser Asp Thr Ala Gly Leu Lys Asn Val Val Ala Thr Met Gly Thr Gln
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Leu Ser Asp Glu His Ile Thr Phe Ile Arg Lys Leu Thr Ser Asn Ile
 260 265 270

Thr Leu Met Phe Asp Gly Asp Phe Ala Gly Ser Glu Ala Thr Leu Lys
 275 280 285

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Thr Gly Gln His Leu Leu Gln Gln Gly Leu Asn Val Phe Val Ile Gln
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Leu Pro Ser Gly Met Asp Pro Asp Glu Tyr Ile Gly Lys Tyr Gly Asn
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Asp Ala Phe Thr Thr Phe Val Lys Asn Asp Lys Lys Ser Phe Ala His
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Tyr Lys Val Ser Ile Leu Lys Asp Glu Ile Ala His Asn Asp Leu Ser
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Tyr Glu Arg Tyr Leu Lys Glu Leu Ser His Asp Ile Ser Leu Met Lys
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Ser Ser Ile Leu Gln Gln Lys Ala Ile Asn Asp Val Ala Pro Phe Phe
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Asn Val Ser Pro Glu Gln Leu Ala Asn Glu Ile Gln Phe Asn Gln Ala
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Pro Ala Asn Tyr Tyr Pro Glu Asp Glu Tyr Gly Gly Tyr Asp Glu Tyr
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Leu Ser Arg Arg Glu Lys Ala Glu Arg Ala Phe Leu Lys His Leu Met
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Arg Asp Lys Asp Thr Phe Leu Asn Tyr Tyr Glu Ser Val Asp Lys Asp
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Val Asn Ser Asn Glu Leu Arg Glu Thr Leu Ile Ser Leu Glu Gln Tyr
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Val Ile Asn Glu Lys Gly Gln Glu Thr Ile Glu Ser Leu Asn His Lys
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<212> DNA

<213> Streptococcus pyogenes

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<213> Streptococcus pyogenes

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 Arg Thr Phe Glu Ala Ala Asp Ile Lys Val Thr Phe Asp Ile Gln Ala
 65 70 75 80
 Ala Gln Val Asp Tyr Ser Asp Asp Leu Leu Gln Ala Tyr Tyr Gln Glu
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 100 105 110
 Ser Lys Leu Lys Val Thr Tyr Glu Asp Asp Lys Leu Ile Ile Ala Ala
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 Pro Gly Phe Val Asn Asn Asp His Phe Arg Asn Asn His Leu Pro Asn
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 245 250 255

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Arg	Lys	Phe	Asp	Met	Ile	Ala	Lys	Gly	Ala	Trp	Leu	Arg	Val	Gln	Gly	290	295	300	
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Gln	Val	Lys	Glu	Ile	Val	Arg	His	Glu	Arg	Lys	Asp	Leu	Met	Pro	Glu	325	330	335	
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Asp	Ala	Leu	Pro	Thr	Val	Glu	Ser	Leu	Ile	Asp	Thr	Ala	Ala	Lys	Trp	355	360	365	
Gly	His	Lys	Ala	Ile	Ala	Ile	Thr	Asp	His	Ala	Asn	Val	Gln	Ser	Phe	370	375	380	
Pro	His	Gly	Tyr	His	Arg	Ala	Arg	Lys	Ala	Gly	Ile	Lys	Ala	Ile	Phe	385	390	395	400
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Pro	Val	Asp	Met	Asp	Leu	His	Glu	Ala	Thr	Tyr	Val	Val	Phe	Asp	Val	420	425	430	
Glu	Thr	Thr	Gly	Leu	Ser	Ala	Met	Asn	Asn	Asp	Leu	Ile	Gln	Ile	Ala	435	440	445	
Ala	Ser	Lys	Met	Phe	Lys	Gly	Asn	Ile	Val	Glu	Gln	Phe	Asp	Glu	Phe	450	455	460	
Ile	Asp	Pro	Gly	His	Pro	Leu	Ser	Ala	Phe	Thr	Thr	Glu	Leu	Thr	Gly	465	470	475	480
Ile	Thr	Asp	Lys	His	Leu	Gln	Gly	Ala	Lys	Pro	Leu	Val	Thr	Val	Leu	485	490	495	
Lys	Ala	Phe	Gln	Asp	Phe	Cys	Lys	Asp	Ser	Ile	Leu	Val	Ala	His	Asn	500	505	510	

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Ala	Ser	Phe	Asp	Val	Gly	Phe	Met	Asn	Ala	Asn	Tyr	Glu	Arg	His	Asp
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Lys	His	Gly	Ile	Lys	Asn	Leu	Leu	Gln	Leu	Asn	Thr	Asp	Leu	Val	Ala
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Glu Val Asp Arg Leu Ala Ala Gly Ala Ala Gly Val Lys Arg Thr Thr
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Gly Gln His Pro Gly Gly Ile Val Val Ile Pro Asn Tyr Met Asp Val
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Tyr Asp Phe Thr Pro Val Gln Tyr Pro Ala Asp Asp Val Thr Ala Ser
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Trp Gln Thr Thr His Phe Asn Phe His Asp Ile Asp Glu Asn Val Leu
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Lys Leu Asp Ile Leu Gly His Asp Asp Pro Thr Met Ile Arg Lys Leu
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Leu Gly Asn Ala Gln Asp Leu Ile Lys Glu Gly Ile Ala Thr Leu Lys
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Thr Val Ile Gly Cys Arg Asp Asp Ile Met Val Tyr Leu Met His Ala
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Gly Leu Glu Pro Lys Met Ala Phe Thr Ile Met Glu Arg Val Arg Lys
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Gly Leu Trp Leu Lys Ile Ser Glu Glu Glu Arg Asn Gly Tyr Ile Asp
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Ala Met Arg Glu Asn Asn Val Pro Asp Trp Tyr Ile Glu Ser Cys Gly
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Lys Ile Lys Tyr Met Phe Pro Lys Ala His Ala Ala Ala Tyr Val Leu
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Met Ala Leu Arg Val Ala Tyr Phe Lys Val His His Pro Ile Met Tyr
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Asn Val Ala Lys Gln Ile Val Lys Ala Arg Gln Glu Gly Glu Phe Leu
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Ser Lys Met Glu Leu Arg Lys Arg Gly Gly Ala Ser Ser Thr Leu Val
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<212> DNA

<213> Streptococcus pyogenes

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<211> 1034

<212> PRT

<213> Streptococcus pyogenes

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Lys	Met	Ser	Gly	Lys	Leu	His	Met	Asp	Tyr	Phe	Cys	Gln	His	Leu	Glu
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Val	Pro	Phe	Asp	Tyr	Tyr	Met	Gly	Val	Asp	Gln	Tyr	Thr	Asp	Leu	Ser
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His	Met	Asp	Ser	Lys	Arg	Gln	Leu	Ile	Pro	Leu	Arg	Thr	Val	Arg	Tyr
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Leu	Ala	Asp	Cys	Gln	Gln	Leu	Thr	Ala	Phe	Tyr	Gln	Thr	His	Cys	Pro
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 Arg Tyr Ser Met Pro Asp Ile Asp Ile Asp Leu Pro Asp Ile Tyr Arg
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 370 375 380
 Asp Val Phe Lys Arg Phe Gly Val Pro Glu Tyr Glu Leu Thr Asn Leu
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 Thr Lys Lys Ile Gly Phe Lys Asp Ser Leu Ala Thr Val Tyr Glu Lys
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 Ser Ile Ser Phe Arg Gln Val Ile Asn Ser Arg Thr Glu Phe Gln Lys
 420 425 430
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Arg Asn Leu Thr Phe Val Gln Lys Met Gln Glu Lys Val Ala Lys Asp
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Tyr Gly Cys Gln Ile Asp Ile Thr Ala Ile Asp Leu Glu Asp Pro Gln
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Thr Leu Ala Leu Phe Ala Lys Gly Asp Thr Lys Gly Ile Phe Gln Phe
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Glu Gln Asn Gly Ala Ile Asn Leu Leu Lys Arg Ile Lys Pro Gln Arg
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Phe Glu Glu Ile Val Ala Thr Thr Ser Leu Asn Arg Pro Gly Ala Ser
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Asp Tyr Thr Thr Asn Phe Ile Lys Arg Arg Glu Gly Gln Glu Lys Ile
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Asp Leu Ile Asp Pro Val Ile Ala Pro Ile Leu Glu Pro Thr Tyr Gly
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Ile Met Leu Tyr Gln Glu Gln Val Met Gln Ile Ala Gln Val Tyr Ala
 610 615 620

Gly Phe Thr Leu Gly Lys Ala Asp Leu Leu Arg Arg Ala Met Ser Lys
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Lys Asn Leu Gln Glu Met Gln Lys Met Glu Glu Asp Phe Ile Ala Ser
 645 650 655

Ala Lys His Leu Gly Arg Ala Glu Glu Thr Ala Arg Gly Leu Phe Lys
 660 665 670

Arg Met Glu Lys Phe Ala Gly Tyr Gly Phe Asn Arg Ser His Ala Phe
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Ala Tyr Ser Ala Leu Ala Phe Gln Leu Ala Tyr Phe Lys Ala His Tyr
 690 695 700

Pro Ala Val Phe Tyr Asp Ile Met Met Asn Tyr Ser Ser Ser Asp Tyr
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Ile Thr Asp Ala Leu Glu Ser Asp Phe Gln Val Ala Gln Val Thr Ile
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755	760	765
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770	775	780
Pro Glu Lys Tyr Gln Lys Lys Val Phe Leu Glu Pro Leu Ile Lys Ile		
785	790	795 800
Gly Leu Phe Asp Cys Phe Glu Pro Asn Arg Lys Lys Ile Leu Asp Asn		
805	810	815
Leu Asp Gly Leu Leu Val Phe Val Asn Glu Leu Gly Ser Leu Phe Ser		
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Asp Ser Ser Phe Ser Trp Val Asp Thr Lys Asp Tyr Ser Val Thr Glu		
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Lys Tyr Ser Leu Glu Gln Glu Ile Val Gly Val Gly Met Ser Lys His		
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Pro Leu Ile Asp Ile Ala Glu Lys Ser Thr Gln Thr Phe Thr Pro Ile		
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Ser Gln Leu Val Lys Glu Ser Glu Ala Val Val Leu Ile Gln Ile Asp		
885	890	895
Ser Ile Arg Ile Ile Arg Thr Lys Thr Ser Gly Gln Gln Met Ala Phe		
900	905	910
Leu Ser Val Asn Asp Thr Lys Lys Lys Leu Asp Val Thr Leu Phe Pro		
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Gln Glu Tyr Ala Ile Tyr Lys Asp Gln Leu Lys Glu Gly Glu Phe Tyr		
930	935	940
Tyr Leu Lys Gly Arg Ile Lys Glu Arg Asp His Arg Leu Gln Met Val		
945	950	955 960
Cys Gln Gln Val Gln Met Ala Ile Ser Gln Lys Tyr Trp Leu Leu Val		
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Glu Asn His Gln Phe Asp Ser Gln Ile Ser Glu Ile Leu Gly Ala Phe		
980	985	990

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Pro Gly Thr Thr Pro Val Val Ile His Tyr Gln Lys Asn Lys Glu Thr
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Lys Ser Arg Leu Met Glu Gln Ile Ala Phe Asp Lys Asp Asp Leu Ala
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 Tyr Ser Tyr Phe Asp Met Ser Glu Ala Ala Tyr Gln Asp Ala Glu Met
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 Asp Leu Val Ser Leu Pro Phe Phe Ala Glu Gln Lys Val Val Ile Phe
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 Asp His Leu Leu Asp Ile Thr Thr Asn Lys Lys Ser Phe Leu Lys Glu
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 Lys Asp Leu Lys Ala Phe Glu Ala Tyr Leu Glu Asn Pro Leu Glu Thr
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 Thr Arg Leu Ile Ile Phe Ala Pro Gly Lys Leu Asp Ser Lys Arg Arg
 115 120 125
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 Leu Lys Glu Ala Glu Leu Arg Thr Tyr Phe Gln Lys Tyr Ser His Gln
 145 150 155 160
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 Ser Asn Asp Asp Phe Ser Gln Ile Met Lys Asn Met Ala Phe Leu Lys
 180 185 190
 Ala Tyr Lys Lys Thr Gly Asn Ile Ser Leu Thr Asp Ile Glu Gln Ala
 195 200 205
 Ile Pro Lys Ser Leu Gln Asp Asn Ile Phe Asp Leu Thr Arg Leu Val
 210 215 220
 Leu Gly Gly Lys Ile Asp Ala Ala Arg Asp Leu Ile His Asp Leu Arg
 225 230 235 240
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 245 250 255
 Phe Arg Leu Phe Leu Gln Leu Thr Ile Leu Ala Arg Asp Val Lys Asn
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 Glu Gln Gln Leu Val Ile Ser Leu Ser Asp Ile Leu Gly Arg Arg Val
 275 280 285

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Asn Pro Tyr Gln Val Lys Tyr Ala Leu Lys Asp Ser Arg Thr Leu Ser
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Leu Ala Phe Leu Thr Gly Ala Val Lys Thr Leu Ile Glu Thr Asp Tyr
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<212> PRT

<213> Streptococcus pyogenes

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Leu Ile Glu Gln Gly Asp Phe Ala Asp Val Thr Val Leu Glu Pro Thr
 65 70 75 80

Gly Gln Val Ile Lys Thr Asp Val Val Lys Glu Met Met Ala Asn Phe
 85 90 95

Ser Gln Thr Gly Tyr Glu Asn Lys Arg Gln Val Phe Ile Ile Lys Asp
 100 105 110

Cys Asp Lys Met His Ile Asn Ala Ala Asn Ser Leu Leu Lys Tyr Ile
 115 120 125

Glu Glu Pro Gln Gly Glu Ala Tyr Ile Phe Leu Leu Thr Asn Asp Asp
 130 135 140

Asn Lys Val Leu Pro Thr Ile Lys Ser Arg Thr Gln Val Phe Gln Phe
 145 150 155 160

Pro Lys Asn Glu Ala Tyr Leu Tyr Gln Leu Ala Gln Glu Lys Gly Leu
 165 170 175

Leu Asn His Gln Ala Lys Leu Val Ala Lys Leu Ala Thr Asn Thr Ser
 180 185 190

His Leu Glu Arg Leu Leu Gln Thr Ser Lys Leu Leu Glu Leu Ile Thr
 195 200 205

Gln Ala Glu Arg Phe Val Ser Ile Trp Leu Lys Asp Gln Leu Gln Ala
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Tyr Leu Ala Leu Asn Arg Leu Val Gln Leu Ala Thr Glu Lys Glu Glu
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Gln Asp Leu Val Leu Thr Leu Leu Thr Leu Leu Leu Ala Arg Glu Arg
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Ala Gln Thr Pro Leu Thr Gln Leu Glu Ala Val Tyr Gln Ala Arg Leu
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Met Trp Gln Ser Asn Val Asn Phe Gln Asn Thr Leu Glu Tyr Met Val
 275 280 285

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Met Ser Glu
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<211> 1665

<212> DNA

<213> *Streptococcus pyogenes*

<400> 25

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<211> 555

<212> PRT

<213> *Streptococcus pyogenes*

<400> 26

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Ser Gly Lys Ile Ser His Ala Tyr Leu Phe Ser Gly Pro Arg Gly Thr	35	40	45
Gly Lys Thr Ser Ala Ala Lys Ile Phe Ala Lys Ala Met Asn Cys Pro	50	55	60
Asn Gln Val Asp Gly Glu Pro Cys Asn Gln Cys Asp Ile Cys Arg Asp	65	70	75
Ile Thr Asn Gly Ser Leu Glu Asp Val Ile Glu Ile Asp Ala Ala Ser	85	90	95
Asn Asn Gly Val Asp Glu Ile Arg Asp Ile Arg Asp Lys Ser Thr Tyr	100	105	110
Ala Pro Ser Arg Ala Thr Tyr Lys Val Tyr Ile Ile Asp Glu Val His	115	120	125
Met Leu Ser Thr Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu Glu	130	135	140
Pro Thr Glu Asn Val Phe Ile Leu Ala Thr Thr Glu Leu His Lys Ile	145	150	155
Pro Ala Thr Ile Leu Ser Arg Val Gln Arg Phe Glu Phe Lys Ala Ile	165	170	175
Lys Gln Lys Ala Ile Arg Glu His Leu Ala Trp Val Leu Asp Lys Glu	180	185	190
Gly Ile Ala Tyr Glu Val Asp Ala Leu Asn Leu Ile Ala Arg Arg Ala	195	200	205
Glu Gly Gly Met Arg Asp Ala Leu Ser Ile Leu Asp Gln Ala Leu Ser	210	215	220
Leu Ser Pro Asp Asn Gln Val Ala Ile Ala Ile Ala Glu Glu Ile Thr	225	230	235
Gly Ser Ile Ser Ile Leu Ala Leu Gly Asp Tyr Val Arg Tyr Val Ser	245	250	255
Gln Glu Gln Ala Thr Gln Ala Leu Ala Ala Leu Glu Thr Ile Tyr Asp			

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260	265	270
Ser Gly Lys Ser Met Ser Arg Phe Ala Thr Asp Leu Leu Thr Tyr Leu		
275	280	285
Arg Asp Leu Leu Val Val Lys Ala Gly Gly Asp Asn Gln Arg Gln Ser		
290	295	300
Ala Val Phe Asp Thr Asn Leu Ser Leu Ser Ile Asp Arg Ile Phe Gln		
305	310	315 320
Met Ile Thr Val Val Thr Ser His Leu Pro Glu Ile Lys Lys Gly Thr		
	325 330	335
His Pro Arg Ile Tyr Ala Glu Met Met Thr Ile Gln Leu Ala Gln Lys		
	340 345	350
Glu Gln Ile Leu Ser Gln Val Asn Leu Ser Gly Glu Leu Ile Ser Glu		
	355 360	365
Ile Glu Thr Leu Lys Asn Glu Leu Ala Gln Leu Lys Gln Gln Leu Ser		
	370 375	380
Gln Leu Gln Ser Arg Pro Asp Ser Leu Ala Arg Ser Asp Lys Thr Lys		
385	390 395	400
Pro Lys Thr Thr Ser Tyr Arg Val Asp Arg Val Thr Ile Leu Lys Ile		
	405 410	415
Met Glu Glu Thr Val Arg Asn Ser Gln Gln Ser Arg Gln Tyr Leu Asp		
	420 425	430
Ala Leu Lys Asn Ala Trp Asn Glu Ile Leu Asp Asn Ile Ser Ala Gln		
	435 440	445
Asp Arg Ala Leu Leu Met Gly Ser Glu Pro Val Leu Ala Asn Ser Glu		
	450 455	460
Asn Ala Ile Leu Ala Phe Glu Ala Ala Phe Asn Ala Glu Gln Val Met		
465	470 475	480
Ser Arg Asn Asn Leu Asn Asp Met Phe Gly Asn Ile Met Ser Lys Ala		
	485 490	495
Ala Gly Phe Ser Pro Asn Ile Leu Ala Val Pro Arg Thr Asp Phe Gln		
	500 505	510
His Ile Arg Lys Glu Phe Ala Gln Glp Met Lys Ser Gln Lys Asp Ser		

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515	520	525
Val Gln Glu Glu Gln Glu Val Ala Leu Asp Ile Pro Glu Gly Phe Asp		
530	535	540
Phe Leu Leu Asp Lys Ile Asn Thr Ile Asp Asp		
545	550	555

<210> 27
 <211> 1134
 <212> DNA
 <213> Streptococcus pyogenes

<400> 27
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 acaggagtaa ctttaacagg gtctaacggc caaatatcaa ttgaaaacac tattcctgta 180
 agtaatgaaa atgctgggtt gctaattacc tctccaggag ctattttatt agaagctagt 240
 ttttttatta atattatttc aagtttgcca gatattagta taaatgttaa agaaattgaa 300
 caacaccaag ttgttttaac cagtggtaaa tcagagatta ccttaaaagg aaaagatgtt 360
 gaccagtatc ctcgctctaca agaagtatca acagaaaatc ctttgatttt aaaaacaaaa 420
 ttattgaagt ctattattgc tgaaacagct tttgcagcca gtttacaaga aagtcgtcct 480
 attttaacag gagttcatat tgtattaagt aatcataaag attttaaagc agtagcgact 540
 gactctcatc gtatgagcca acgtttaatc actttggaca atacttcagc agatttgatg 600
 gtagttcttc caagtaaatc tttgagagaa ttttcagcag tatttacaga tgatattgag 660
 accgttgagg tatttttctc accaagccaa atcttggtca gaagtgaaca catttctttt 720
 tatacacgcc tcttagaagg aaattatccc gatacagacc gtttattaat gacagaattt 780
 gagacggagg ttgttttcaa tacccaatcc cttcgccacg ctatggaacg tgccttcttg 840
 atttctaatt ctactcaaaa tgggtactgtt aagcttgaga ttactcaaaa tcatatttca 900
 gctcatgtta actcacctga ggttggttaag gtaaacgagg atttagatat tgtagtcag 960
 tctggttagtg atttaactat cagcttcaat ccaacttacc ttattgagtc tttaaaagct 1020
 attaaaagtg aaacagtaaa aattcatttc ttatcaccag ttcgaccatt caccctaaca 1080
 ccaggcgatg aggaagaaag ttttatccaa ttaattacac cagtacgaac aaac 1134

<210> 28
 <211> 378
 <212> PRT
 <213> Streptococcus pyogenes

<400> 28
 Met Ile Gln Phe Ser Ile Asn Arg Thr Leu Phe Ile His Ala Leu Asn
 1 5 10 15
 Thr Thr Lys Arg Ala Ile Ser Thr Lys Asn Ala Ile Pro Ile Leu Ser
 20 25 30

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Ser	Ile	Lys	Ile	Glu	Val	Thr	Ser	Thr	Gly	Val	Thr	Leu	Thr	Gly	Ser	35	40	45	
Asn	Gly	Gln	Ile	Ser	Ile	Glu	Asn	Thr	Ile	Pro	Val	Ser	Asn	Glu	Asn	50	55	60	
Ala	Gly	Leu	Leu	Ile	Thr	Ser	Pro	Gly	Ala	Ile	Leu	Leu	Glu	Ala	Ser	65	70	75	80
Phe	Phe	Ile	Asn	Ile	Ile	Ser	Ser	Leu	Pro	Asp	Ile	Ser	Ile	Asn	Val	85	90	95	
Lys	Glu	Ile	Glu	Gln	His	Gln	Val	Val	Leu	Thr	Ser	Gly	Lys	Ser	Glu	100	105	110	
Ile	Thr	Leu	Lys	Gly	Lys	Asp	Val	Asp	Gln	Tyr	Pro	Arg	Leu	Gln	Glu	115	120	125	
Val	Ser	Thr	Glu	Asn	Pro	Leu	Ile	Leu	Lys	Thr	Lys	Leu	Leu	Lys	Ser	130	135	140	
Ile	Ile	Ala	Glu	Thr	Ala	Phe	Ala	Ala	Ser	Leu	Gln	Glu	Ser	Arg	Pro	145	150	155	160
Ile	Leu	Thr	Gly	Val	His	Ile	Val	Leu	Ser	Asn	His	Lys	Asp	Phe	Lys	165	170	175	
Ala	Val	Ala	Thr	Asp	Ser	His	Arg	Met	Ser	Gln	Arg	Leu	Ile	Thr	Leu	180	185	190	
Asp	Asn	Thr	Ser	Ala	Asp	Leu	Met	Val	Val	Leu	Pro	Ser	Lys	Ser	Leu	195	200	205	
Arg	Glu	Phe	Ser	Ala	Val	Phe	Thr	Asp	Asp	Ile	Glu	Thr	Val	Glu	Val	210	215	220	
Phe	Phe	Ser	Pro	Ser	Gln	Ile	Leu	Phe	Arg	Ser	Glu	His	Ile	Ser	Phe	225	230	235	240
Tyr	Thr	Arg	Leu	Leu	Glu	Gly	Asn	Tyr	Pro	Asp	Thr	Asp	Arg	Leu	Leu	245	250	255	
Met	Thr	Glu	Phe	Glu	Thr	Glu	Val	Val	Phe	Asn	Thr	Gln	Ser	Leu	Arg	260	265	270	
His	Ala	Met	Glu	Arg	Ala	Phe	Leu	Ile	Ser	Asn	Ala	Thr	Gln	Asn	Gly	275	280	285	

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Thr Val Lys Leu Glu Ile Thr Gln Asn His Ile Ser Ala His Val Asn
 290 295 300

Ser Pro Glu Val Gly Lys Val Asn Glu Asp Leu Asp Ile Val Ser Gln
 305 310 315 320

Ser Gly Ser Asp Leu Thr Ile Ser Phe Asn Pro Thr Tyr Leu Ile Glu
 325 330 335

Ser Leu Lys Ala Ile Lys Ser Glu Thr Val Lys Ile His Phe Leu Ser
 340 345 350

Pro Val Arg Pro Phe Thr Leu Thr Pro Gly Asp Glu Glu Glu Ser Phe
 355 360 365

Ile Gln Leu Ile Thr Pro Val Arg Thr Asn
 370 375

<210> 29

<211> 492

<212> DNA

<213> Streptococcus pyogenes

<400> 29

atgattaata atgtagtact agttggctgc atgaccaagg atgcagaact tcgttacaca 60
 ccaagtcaag tagctgtggc taccttcaca cttgctgtta accgtacctt taaaagccaa 120
 aatggtgaac gcgaggcaga ttccattaac tgtgtgatct ggcgtcaacc ggctgaaaat 180
 ttagcgaaact gggctaaaaa aggtgctttg atcggagtta cgggtcgtat tcatacacgt 240
 aactacgaaa accaacaagg acaacgtgtc tatgtaacag aagttgttgc agataatttc 300
 caaatgttgg aaagtcgtgc tacacgtgaa ggtggctcaa ctgggtcatt taatggtggg 360
 tttacaata acacttcac atcaaacagt tactcagcgc ctgcacaaca aacgcctaac 420
 tttggaagag atgatagccc atttgggaac tcaaaccga tggatatctc agatgacgat 480
 cttccattct ag 492

<210> 30

<211> 163

<212> PRT

<213> Streptococcus pyogenes

<400> 30

Met Ile Asn Asn Val Val Leu Val Gly Arg Met Thr Lys Asp Ala Glu
 1 5 10 15

Leu Arg Tyr Thr Pro Ser Gln Val Ala Val Ala Thr Phe Thr Leu Ala
 20 25 30

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Val Asn Arg Thr Phe Lys Ser Gln Asn Gly Glu Arg Glu Ala Asp Phe
 35 40 45

Ile Asn Cys Val Ile Trp Arg Gln Pro Ala Glu Asn Leu Ala Asn Trp
 50 55 60

Ala Lys Lys Gly Ala Leu Ile Gly Val Thr Gly Arg Ile Gln Thr Arg
 65 70 75 80

Asn Tyr Glu Asn Gln Gln Gly Gln Arg Val Tyr Val Thr Glu Val Val
 85 90 95

Ala Asp Asn Phe Gln Met Leu Glu Ser Arg Ala Thr Arg Glu Gly Gly
 100 105 110

Ser Thr Gly Ser Phe Asn Gly Gly Phe Asn Asn Asn Thr Ser Ser Ser
 115 120 125

Asn Ser Tyr Ser Ala Pro Ala Gln Gln Thr Pro Asn Phe Gly Arg Asp
 130 135 140

Asp Ser Pro Phe Gly Asn Ser Asn Pro Met Asp Ile Ser Asp Asp Asp
 145 150 155 160

Leu Pro Phe

<210> 31

<211> 1815

<212> DNA

<213> Streptococcus pyogenes

<400> 31

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 aaaaatagcg ttaatatgt cgatgtcatt ggagaagtgg tcaaactttc ccgatcaggg 120
 cggcattacc tcgggctttg cccatttcat aaggaaaaga caccctcttt taatgttggt 180
 gaagacagac aattttttca ctgctttggc tgtggaaaat caggggatgt ttttaaattt 240
 attgaggaat accgccaagt ccccttctta gaaagtgttc agattattgc cgataagact 300
 ggtatgtcgc ttaatatacc gccaaagtcag gcagtacttg ctagccaaca caagcaccct 360
 aatcacgctt tgatgacact tcatgaggat gctgctaaat tttaccatgc agttttgatg 420
 accactacca ttggtcaaga agctaggaag tacctttacc agagaggctt ggatgaccaa 480
 ttaattgagc atttcaatat tggtttagcc ccagatgagt cagattatct ttatcaagct 540
 ctttctaaaa aatacgagga aggtcaattg gttgcttcag gattgtttca cttgtccgat 600
 caatccaata ccatttacga cgcctttcga aatcgatca tgtttccctt atcagatgac 660
 cgagggcata ttattgcctt ttcaggacgt atctggacgg cagctgatat ggaaaagaga 720
 caggcaaagt ataaaaatc aagaggaaca gttcttttta acaaacttta tgaattgtat 780
 catctggaca aggcaaggcc tgttattgcc aaaacccatg aagtgtttct aatggaaggg 840

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```

tttatggacg tgattgccgc ttaccgttcc ggctatgaaa atgctgttgc ttcaatgggg 900
acggcattga ctcaagaaca tgtcaatcac cttaagcaag tcactaaaaa agttgttttg 960
atztatgatg gtgacgatgc tggacaacat gctattgcaa aatcactaga attgcttaaa 1020
gattttgttg tcgaaattgt cagaatcccc aataaaatgg atcctgacga atttgtacaa 1080
cggcattccc cagaagcatt tgcagatttg cttaagcagt cacggatcag tagtggtgaa 1140
ttttttattg attacctaaa acctactaat gtagacaatt tgcaatcaca aattgtttat 1200
gtggagaaaa tggcaccatt gattgctcaa tcaccatcca tcacagctca acattcgtat 1260
attaacaaga ttgctgattt gttgccaaac tttgactatt ttcaagtaga acaatcagta 1320
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aatcttgtga ccttaccaat gccaaaaagt ttgacagcta ttgctaagac agaaagtcac 1440
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ttttattttg atacctctac cttagaatta ctttatcaac ggctgaagca acaaggacac 1560
attacatctt atgatttgtc agagatgtca gaggaagtta accgtgctta ttacaatgtt 1620
ttagaagaaa accttcccaa agaagtagct cttgggtgaga ttgatgatat tttatccaaa 1680
cgtgccaaac ttttagcaga gcgcgatctt cacaacaag ggaaaaaagt tagagaatct 1740
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cgaaaaatgg aatag                                     1815

```

<210> 32

<211> 600

<212> PRT

<213> Streptococcus pyogenes

<400> 32

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Met Gly Phe Leu Trp Gly Gly Asp Asp Leu Ala Ile Asp Lys Glu Met
  1                   5                   10                   15

```

```

Ile Ser Gln Val Lys Asn Ser Val Asn Ile Val Asp Val Ile Gly Glu
                20                   25                   30

```

```

Val Val Lys Leu Ser Arg Ser Gly Arg His Tyr Leu Gly Leu Cys Pro
                35                   40                   45

```

```

Phe His Lys Glu Lys Thr Pro Ser Phe Asn Val Val Glu Asp Arg Gln
                50                   55                   60

```

```

Phe Phe His Cys Phe Gly Cys Gly Lys Ser Gly Asp Val Phe Lys Phe
                65                   70                   75                   80

```

```

Ile Glu Glu Tyr Arg Gln Val Pro Phe Leu Glu Ser Val Gln Ile Ile
                85                   90                   95

```

```

Ala Asp Lys Thr Gly Met Ser Leu Asn Ile Pro Pro Ser Gln Ala Val
                100                   105                   110

```

```

Leu Ala Ser Gln His Lys His Pro Asn His Ala Leu Met Thr Leu His
                115                   120                   125

```

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Glu Asp Ala Ala Lys Phe Tyr His Ala Val Leu Met Thr Thr Thr Ile
 130 135 140

Gly Gln Glu Ala Arg Lys Tyr Leu Tyr Gln Arg Gly Leu Asp Asp Gln
 145 150 155 160

Leu Ile Glu His Phe Asn Ile Gly Leu Ala Pro Asp Glu Ser Asp Tyr
 165 170 175

Leu Tyr Gln Ala Leu Ser Lys Lys Tyr Glu Glu Gly Gln Leu Val Ala
 180 185 190

Ser Gly Leu Phe His Leu Ser Asp Gln Ser Asn Thr Ile Tyr Asp Ala
 195 200 205

Phe Arg Asn Arg Ile Met Phe Pro Leu Ser Asp Asp Arg Gly His Ile
 210 215 220

Ile Ala Phe Ser Gly Arg Ile Trp Thr Ala Ala Asp Met Glu Lys Arg
 225 230 235 240

Gln Ala Lys Tyr Lys Asn Ser Arg Gly Thr Val Leu Phe Asn Lys Ser
 245 250 255

Tyr Glu Leu Tyr His Leu Asp Lys Ala Arg Pro Val Ile Ala Lys Thr
 260 265 270

His Glu Val Phe Leu Met Glu Gly Phe Met Asp Val Ile Ala Ala Tyr
 275 280 285

Arg Ser Gly Tyr Glu Asn Ala Val Ala Ser Met Gly Thr Ala Leu Thr
 290 295 300

Gln Glu His Val Asn His Leu Lys Gln Val Thr Lys Lys Val Val Leu
 305 310 315 320

Ile Tyr Asp Gly Asp Asp Ala Gly Gln His Ala Ile Ala Lys Ser Leu
 325 330 335

Glu Leu Leu Lys Asp Phe Val Val Glu Ile Val Arg Ile Pro Asn Lys
 340 345 350

Met Asp Pro Asp Glu Phe Val Gln Arg His Ser Pro Glu Ala Phe Ala
 355 360 365

Asp Leu Leu Lys Gln Ser Arg Ile Ser Ser Val Glu Phe Phe Ile Asp
 370 375 380

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Tyr Leu Lys Pro Thr Asn Val Asp Asn Leu Gln Ser Gln Ile Val Tyr
 385 390 395 400

Val Glu Lys Met Ala Pro Leu Ile Ala Gln Ser Pro Ser Ile Thr Ala
 405 410 415

Gln His Ser Tyr Ile Asn Lys Ile Ala Asp Leu Leu Pro Asn Phe Asp
 420 425 430

Tyr Phe Gln Val Glu Gln Ser Val Asn Ala Leu Arg Ile Gln Asp Arg
 435 440 445

Gln Lys His Gln Gly Gln Ile Ala Gln Ala Val Ser Asn Leu Val Thr
 450 455 460

Leu Pro Met Pro Lys Ser Leu Thr Ala Ile Ala Lys Thr Glu Ser His
 465 470 475 480

Leu Met His Arg Leu Leu His His Asp Tyr Leu Leu Asn Glu Phe Arg
 485 490 495

His Arg Asp Asp Phe Tyr Phe Asp Thr Ser Thr Leu Glu Leu Leu Tyr
 500 505 510

Gln Arg Leu Lys Gln Gln Gly His Ile Thr Ser Tyr Asp Leu Ser Glu
 515 520 525

Met Ser Glu Glu Val Asn Arg Ala Tyr Tyr Asn Val Leu Glu Glu Asn
 530 535 540

Leu Pro Lys Glu Val Ala Leu Gly Glu Ile Asp Asp Ile Leu Ser Lys
 545 550 555 560

Arg Ala Lys Leu Leu Ala Glu Arg Asp Leu His Lys Gln Gly Lys Lys
 565 570 575

Val Arg Glu Ser Ser Asn Lys Gly Asp His Gln Ala Ala Leu Glu Val
 580 585 590

Leu Glu His Phe Ile Ala Gln Lys
 595 600

<210> 33

<211> 1368

<212> DNA

<213> Streptococcus pyogenes

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<400> 33

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agtccagacg atttttataa gtacgctcat aaaatttatct ttcgggcaat gattaccctc 180
agcgatcgta atgatgccat tgatgcaacc actataagaa caatcctaga tgatcaagat 240
gatctgcaaa gtattggttg cttatcctat attgttgaac tagttaatag tgtcccaact 300
agtgctaatt cagaatatta tgctaaaatt gtagctgaga aagctatggt gcgtgatatt 360
attgctaggt tgacagaatc tgtcaaccta gcttatgatg aaattttaaa accagaagag 420
gttatcgctg gagttgagag agctttaatt gaactcaatg aacatagtaa tcgtagtggg 480
tttcgcaaaa tttcagatgt gctaaaagtt aattacgagg ctttagaagc acgttctaag 540
cagacttcaa atgttacagg tttaccaact ggtttttagag accttgacaa gattacaaca 600
ggtttacacc cagatcaatt agttatttta gctgctcggc cagcagtggg gaagactgcc 660
tttgttctta atattgcgca aaatgtgggg actaagcaaa aaaagactgt tgctattttt 720
tccttggaag tgggtgctga aagtttagta gatcgtatgc ttgcagcaga aggaatggtt 780
gattcgcaca gtttaagaac agggcaactc acagatcagg attggaataa tgtaacaatt 840
gctcagggag ctttggcaga agcaccgatt tatattgacg atacgcccg gattaaaatt 900
actgaaatcc gcgcaagatc acggaaattg tctcaagaag tggatggttg tttaggtctc 960
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gtttcagata tttcaagaca gcttaaaatc ctagctaaag aattgaaagt accagttatt 1080
gccctaagtc agctttctcg tggcgttgag caaaggcaag ataaacgacc agttttatca 1140
gatattcgtg aatcaggatc tattgagcag gatgccgata ttgtagcctt cttataccgg 1200
gacgattatt accgtaaaga atgtgatgat gctgaagaag ctggtgaaga taacacaatt 1260
gaagttatcc tcgagaaaaa tagagctggg gcgcgtggaa cagtcaaact gatgttccaa 1320
aaagaataca acaaattctc aagtatagcc cagtttgaag aaagataa 1368

```

<210> 34

<211> 455

<212> PRT

<213> Streptococcus pyogenes

<400> 34

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Met Arg Leu Pro Glu Val Ala Glu Leu Arg Val Gln Pro Gln Asp Leu
  1             5             10             15

```

```

Leu Ala Glu Gln Ser Val Leu Gly Ser Ile Phe Ile Ser Pro Asp Lys
      20             25             30

```

```

Leu Ile Ala Val Arg Glu Phe Ile Ser Pro Asp Asp Phe Tyr Lys Tyr
      35             40             45

```

```

Ala His Lys Ile Ile Phe Arg Ala Met Ile Thr Leu Ser Asp Arg Asn
      50             55             60

```

```

Asp Ala Ile Asp Ala Thr Thr Ile Arg Thr Ile Leu Asp Asp Gln Asp
      65             70             75             80

```

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Asp	Leu	Gln	Ser	Ile	Gly	Gly	Leu	Ser	Tyr	Ile	Val	Glu	Leu	Val	Asn	
				85					90						95	
Ser	Val	Pro	Thr	Ser	Ala	Asn	Ala	Glu	Tyr	Tyr	Ala	Lys	Ile	Val	Ala	
			100					105					110			
Glu	Lys	Ala	Met	Leu	Arg	Asp	Ile	Ile	Ala	Arg	Leu	Thr	Glu	Ser	Val	
		115					120					125				
Asn	Leu	Ala	Tyr	Asp	Glu	Ile	Leu	Lys	Pro	Glu	Glu	Val	Ile	Ala	Gly	
	130					135						140				
Val	Glu	Arg	Ala	Gln	Gly	Ala	Leu	Ala	Glu	Ala	Pro	Ile	Tyr	Ile	Asp	
145					150					155					160	
Asp	Thr	Pro	Gly	Ile	Lys	Ile	Ala	Leu	Ile	Glu	Leu	Asn	Glu	His	Ser	
			165						170					175		
Asn	Arg	Ser	Gly	Phe	Arg	Lys	Ile	Ser	Asp	Val	Leu	Lys	Val	Asn	Tyr	
			180					185						190		
Glu	Ala	Leu	Glu	Ala	Arg	Ser	Lys	Gln	Thr	Ser	Asn	Val	Thr	Gly	Leu	
		195					200						205			
Pro	Thr	Gly	Phe	Arg	Asp	Leu	Asp	Lys	Ile	Thr	Thr	Gly	Leu	His	Pro	
	210					215						220				
Asp	Gln	Leu	Val	Ile	Leu	Ala	Ala	Arg	Pro	Ala	Val	Gly	Lys	Thr	Ala	
225					230					235					240	
Phe	Val	Leu	Asn	Ile	Ala	Gln	Asn	Val	Gly	Thr	Lys	Gln	Lys	Lys	Thr	
			245						250					255		
Val	Ala	Ile	Phe	Ser	Leu	Glu	Met	Gly	Ala	Glu	Ser	Leu	Val	Asp	Arg	
			260					265						270		
Met	Leu	Ala	Ala	Glu	Gly	Met	Val	Asp	Ser	His	Ser	Leu	Arg	Thr	Gly	
		275					280						285			
Gln	Leu	Thr	Asp	Gln	Asp	Trp	Asn	Asn	Val	Thr	Ile	Thr	Glu	Ile	Arg	
		290					295					300				
Ala	Arg	Ser	Arg	Lys	Leu	Ser	Gln	Glu	Val	Asp	Gly	Gly	Leu	Gly	Leu	
305					310					315					320	
Ile	Val	Ile	Asp	Tyr	Leu	Gln	Leu	Ile	Thr	Gly	Thr	Lys	Pro	Glu	Asn	
			325						330						335	

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Arg Gln Gln Glu Val Ser Asp Ile Ser Arg Gln Leu Lys Ile Leu Ala
 340 345 350
 Lys Glu Leu Lys Val Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Gly
 355 360 365
 Val Glu Gln Arg Gln Asp Lys Arg Pro Val Leu Ser Asp Ile Arg Glu
 370 375 380
 Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg
 385 390 395 400
 Asp Asp Tyr Tyr Arg Lys Glu Cys Asp Asp Ala Glu Glu Ala Val Glu
 405 410 415
 Asp Asn Thr Ile Glu Val Ile Leu Glu Lys Asn Arg Ala Gly Ala Arg
 420 425 430
 Gly Thr Val Lys Leu Met Phe Gln Lys Glu Tyr Asn Lys Phe Ser Ser
 435 440 445
 Ile Ala Gln Phe Glu Glu Arg
 450 455

<210> 35

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 35

ggtggttaatt gtcttgcata tgacagagc

29

<210> 36

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 36

agcgattaag tggattgccg gggtgtgatg c

31

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<210> 37

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 37

agcatcacaa cccggcaatc cacttaatcg c

31

<210> 38

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 38

gactacgcc a tgggcattaa ataaatacc

29

<210> 39

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 39

gaagatgcat ataaacgtgc aagacctagt

30

<210> 40

<211> 34

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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 40

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34

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<210> 41
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 <212> DNA
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<223> Description of Artificial Sequence: primer

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36

<210> 42
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 <211> 15
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<400> 43

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 1 5 10 15

<210> 44
 <211> 15
 <212> PRT
 <213> Escherichia coli

<400> 44

Lys Phe Ala Gly Tyr Gly Phe Asn Lys Ser His Ser Ala Ala Tyr
 1 5 10 15

<210> 45
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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

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44

<210> 46

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer

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45

<210> 47

<211> 39

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<223> Description of Artificial Sequence: primer

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42

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<400> 49

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1 5 10

<210> 50

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<210> 51

<211> 38

<212> DNA

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<210> 52

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ccggaattct ggtggttctt ctaatgtttt taataatgc

39

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<400> 53

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38

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33

<210> 55

<211> 29

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29

<210> 56

<211> 32

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gatactcagt atctttctca gatgttttat tc

32

<210> 57

<211> 14

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Asp Leu Ile Ile Val Ala Ala Arg Pro Ser Met Gly Lys Thr

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1

5

10

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<211> 15

<212> PRT

<213> Escherichia coli

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41

<210> 60

<211> 48

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48

<210> 61

<211> 45

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45

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<210> 62

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48

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31

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<211> 69

<212> DNA

<213> Artificial Sequence

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<400> 66

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28

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<210> 69
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<223> Description of Artificial Sequence: primer

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38

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<210> 70

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<210> 73

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33

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<210> 74
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38

<210> 75
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<223> Description of Artificial Sequence: primer

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34

<210> 76
<211> 38
<212> DNA
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<223> Description of Artificial Sequence: primer

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38

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<223> Description of Artificial Sequence: primer

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tcagatttat tcgc

74

<210> 78

<211> 57

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer

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<210> 79

<211> 43

<212> DNA

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<210> 80

<211> 40

<212> DNA

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<400> 80

cgaatagcag cggtcatacc aggatcctcg ccgccactgg 40

<210> 81

<211> 49

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer

<400> 81

COMBINED DECLARATION FOR PATENT
APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
22221/1023

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**DNA REPLICATION PROTEINS OF GRAM POSITIVE BACTERIA AND
THEIR USE TO SCREEN FOR CHEMICAL INHIBITORS**

the specification of which (check only one item below):

- ☐ is attached hereto.
- ☐ was filed as U.S. Patent Application Serial No. _____ on _____ and was amended on _____ (if applicable).
- ☒ was filed as PCT International Application Number **PCT/US00/20666** on **July 28, 2000** and assigned U.S. Patent Application Serial No. **10/048,071**.

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim priority benefits under Title 35, United States Code, § 119 of any application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States listed below and have also identified below any application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (IF PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
U.S.A.	60/146,178	29 July 1999	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
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			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Continued) (Includes Reference to PCT International Applications)	ATTORNEY'S DOCKET NUMBER 22221/1023
------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS			STATUS (Check One)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. **Michael L. Goldman, Registration No. 30,727; Joseph M. Noto, Registration No. 32,163; Gunnar G. Leinberg, Registration No. 35,584; Edwin V. Merkel, Registration No. 40,087; Georgia Evans, Registration No. 44,597; Alice Y. Choi, Registration No. 45,758; Andrew K. Gonsalves, Registration No. 48,145; Noreen L. Connolly, Registration No. 48,987; John Campa, Registration No. 49,014** 9

Send Correspondence to: Michael L. Goldman, Esq. NIXON PEABODY LLP Clinton Square, P.O. Box 31051 Rochester, New York 14603-1051	Direct telephone calls to: Michael L. Goldman (585) 263-1304
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4-00 2 0 4	FULL NAME OF INVENTOR	FAMILY NAME WHIPPLE	FIRST GIVEN NAME Richard	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY Rareton NJ	STATE/FOREIGN COUNTRY New Jersey	COUNTRY OF CITIZENSHIP U.S.A.
	POST OFFICE ADDRESS	P.O. ADDRESS 91 First Avenue	CITY Rareton	STATE & ZIP CODE/COUNTRY New Jersey 08869 U.S.A.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 <i>Mike Gail</i>	SIGNATURE OF INVENTOR 202 <i>[Signature]</i>	SIGNATURE OF INVENTOR 203 <i>Don Sheng</i>
DATE	DATE <i>4/18/02</i>	DATE <i>4/18/02</i>
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE	DATE	DATE
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE

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49

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<210> 83

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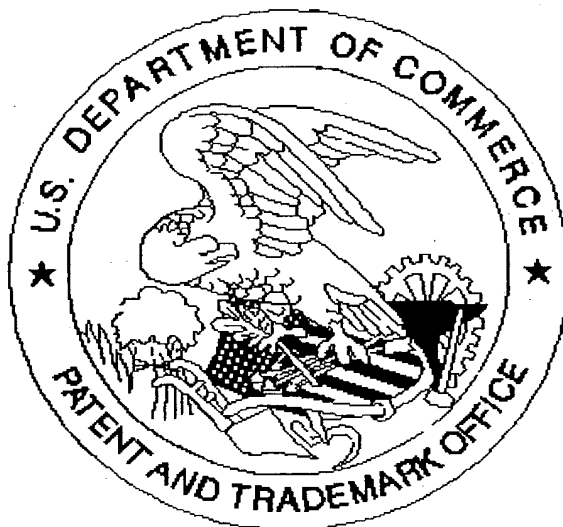
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51

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